











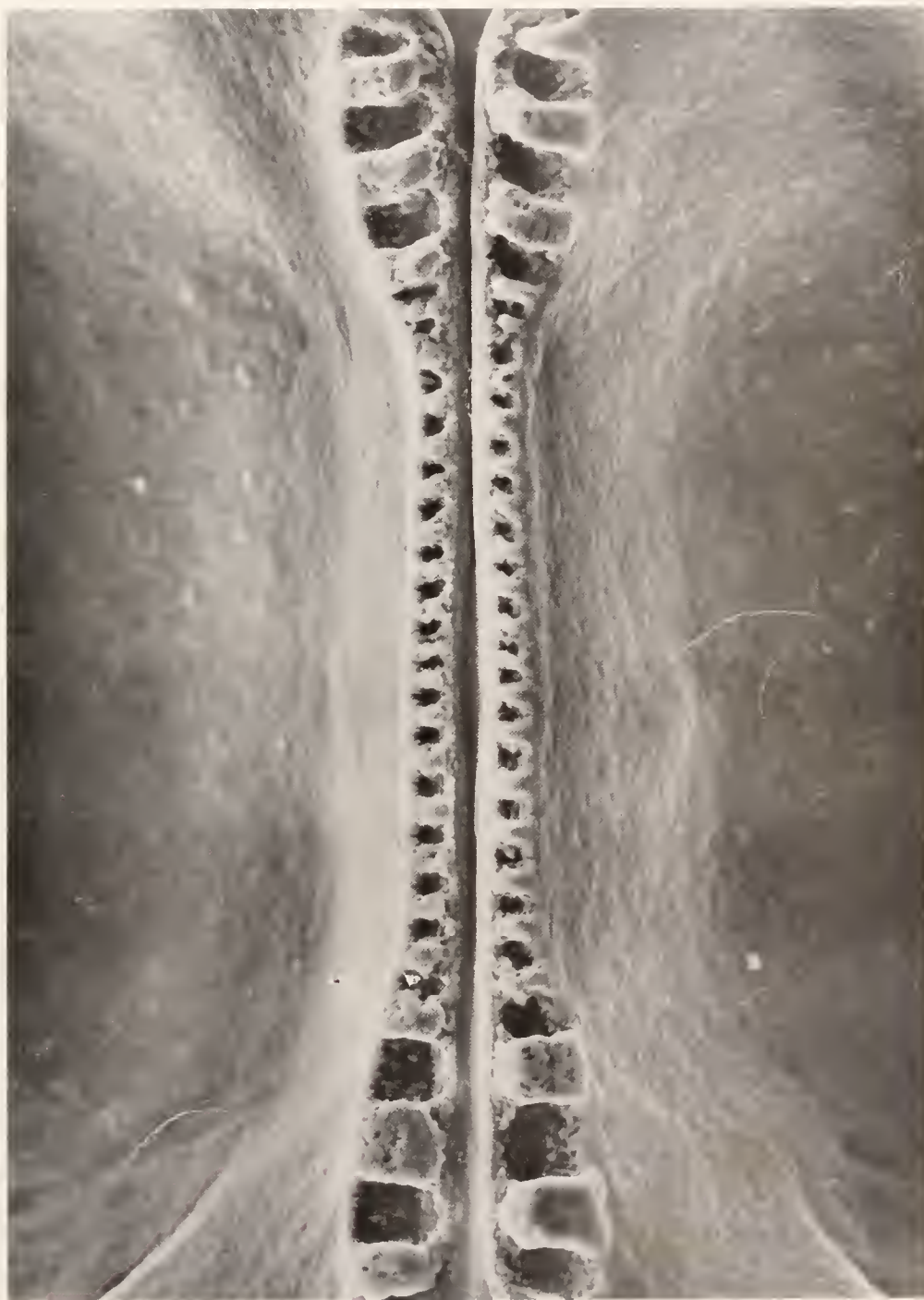




# JOURNAL OF SHELLFISH RESEARCH

VOLUME 2, NUMBER 1

JUNE 1982



*The Journal of Shellfish Research* (formerly *Proceedings of the National Shellfisheries Association*) is the official publication  
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*Journal of Shellfish Research*

Volume 2, Number 1  
June 1982

This issue of the *Journal of Shellfish Research*  
is dedicated to the memory of  
**JAMES BENNETT ENGLE**



AUG 1 2 1983

**JAMES BENNETT ENGLE**, Jim or "Uncle Ben," as his many friends knew him, was born 25 July 1900 in Newark, NJ. He was educated at Newark College of Engineering, and at Columbia and Rutgers universities. During his 35 years of distinguished service as a shellfish biologist, Jim was employed by the U.S. Bureau of Fisheries, the Maryland Department of Natural Resources, the Virginia Fisheries Laboratory, the U.S. Fish and Wildlife Service, the U.S. Bureau of Commercial Fisheries, and the U.S. National Marine Fisheries Service. He served as a Graduate Assistant with Dr. Thurlow C. Nelson; as Assistant Director of the BCF Laboratory at Milford, CT; as Chief of Shellfish Research in Yorktown, VA and Annapolis, MD; and as Director of the NMFS Laboratory at Oxford, MD. He organized and became the first director of the National Shellfish Advisory Service which later became the Sea Grant Marine Advisory Service.

Jim Engle's shellfish interests included oyster predators (drills and seastars) and associate mussels, seed beds and setting, and oyster resources and management. He conducted numerous field investigations from Long Island Sound to Mississippi Sound including Delaware, Chesapeake, and Mobile bays. He published at least 60 technical papers and

reports including 12 in the Proceedings of the National Shellfisheries Association, the forerunner of the *Journal of Shellfish Research*. He received many honors during his lifetime including the Meritorious Service Award of the U.S. Department of the Interior.

Jim Engle was a long-time member of the National Shellfisheries Association (1940–1981). During those 41 years, he seldom missed an annual NSA meeting and served on numerous standing committees. He served as the Association's Vice-President from 1950 to 1952, and as President from 1952 to 1953. Jim was elected to Honorary Membership in 1970.

**JAMES BENNETT ENGLE**, shellfish biologist par excellence, was a man of high integrity and dedication; he was loved and respected by his many, many friends and colleagues. His avocation was working with young people, especially through the Boy Scouts of America. He passed away 23 October 1981 at Easton, MD. He is survived by his wife, Isabel, of Oxford, MD, two daughters, Nancy and Susan, and a great many friends and colleagues in NSA. We have all benefited from his work and presence among us. We all miss him in NSA, but his spirit lives on.

Aaron Rosenfield and Edwin W. Cake, Jr.



# GROWTH, MORTALITY, AND COPPER-NICKEL ACCUMULATION BY OYSTERS (*CRASSOSTREA VIRGINICA*) AT THE MORGANTOWN STEAM ELECTRIC STATION ON THE POTOMAC RIVER, MARYLAND

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**ABSTRACT** Growth of three size classes (initially 20-, 40-, and 80-mm shell height) of the oyster *Crassostrea virginica* (Gmelin) was observed during a 23-month period beginning in December 1976 at the intake and discharge canal areas of the Potomac Electric Power Company's Morgantown generating station located on the Potomac River in Charles County, Maryland, and at a control area in Shady Side, Anne Arundel County, Maryland. A fourth size class (31 mm initial shell height), added to the study in October 1977, was observed for 13 months. In addition, 10 oysters were analyzed monthly from each area for uptake of copper and nickel.

Shell growth of oysters was excellent in all three areas during the 1977 season (salinity  $> 9$  ppt), despite average discharge-canal temperatures  $6^{\circ}\text{C}$  above intake temperatures. Poor shell growth occurred in all three areas during the 1978 season, probably because of low salinity ( $< 6$  ppt). Low salinity during 1978 and high discharge-canal temperatures eventually resulted in near total mortality among discharge-canal oysters. Analysis of regression-generated growth curves revealed that growth of controls was significantly greater ( $p < 0.001$ ) than that of discharge-canal oysters in all four size classes, and growth of intake oysters was significantly greater ( $p \leq 0.002$ ) than that of discharge canal oysters in three of four classes. However, controls grew more than intake oysters in two of four classes ( $p < 0.001$ ), but intake oysters outgrew controls in a third size class ( $p = 0.002$ ).

Metal studies indicated no effect of plant operation on nickel accumulation, but uptake of copper was directly associated with operation. Oysters were able to eliminate much of the accumulated copper within 2 months of transfer to the control area.

## INTRODUCTION

Thermal effluents from electric generating stations may have both beneficial and detrimental effects on oysters. Breeze (1971) showed that a temperature increase of  $13^{\circ}\text{C}$  (from  $12^{\circ}$  to  $25^{\circ}\text{C}$ ) would increase the growth of hatchery-produced seed oysters and thereby ensure survival after planting and possibly shorten the time to harvest. Tinsman and Maurer (1974) and Gilmore et al. (1975) also demonstrated increased shell growth by oysters raised in heated waters, but Tinsman and Maurer (1974) found that the wintertime advantage of oysters maintained in the effluent of the Delmarva power plant in Indian River Bay, Delaware, was partially lost in summer when the oysters had poorer meat condition and suffered higher mortalities than controls.

A major problem of certain power plant effluents is that they contain elevated levels of metals acquired on transit through the plant. Because many generating stations with once-through cooling systems use 70-30 copper-nickel alloy condenser tubes, the uptake of these metals by nearby oysters is a concern. O'Connor (1976) stated that the Chalk Point generating station on the Patuxent River in Maryland discharges 1.8 to 7.3 t (2 to 8 tons) of copper annually to the river. Roosenburg (1969) found high copper concentrations in oysters near the Chalk Point discharge and showed that concentrations decreased as distance from the plant increased. Abbe and Krueger (1977a) and Abbe (1981a) have shown similar results at the Morgantown

power plant on the Potomac River and at the Calvert Cliffs Nuclear Power Plant on Chesapeake Bay, respectively.

This paper presents a study of growth, mortality, and metal accumulation by tray-held oysters in the effluent canal of the Potomac Electric Power Company's (PEPCO) Morgantown generating station, a two-unit fossil-fueled plant producing approximately 1,150 megawatts. The once-through cooling systems uses  $3.8 \times 10^6$   $\ell/\text{min}$  ( $1.0 \times 10^6$  gpm) of Potomac River water which has a temperature increase of up to  $8.3^{\circ}\text{C}$  as it passes through the 70-30 copper-nickel condensers (Guiland 1977). Low-level chlorination to prevent biofouling is continuous during warm months (when river temperature exceeds  $10^{\circ}\text{C}$ ), with each unit receiving about 907 kg/day chlorine. Chlorine residuals are typically 0.02 to 0.05 mg/ $\ell$  at the condenser outlets and decay continues as the water moves along the 630-m discharge canal to the river.

Studies of growth and survival of oysters in this canal are not new. Powell (1973) spent several years determining whether the canal could be used to overwinter very young hatchery seed. He stated that oysters could overwinter there with very high survival, but that they should not be left year-round because the salinity was often too low to allow normal growth. The salinity of the Potomac near Morgantown ranges from less than 5 ppt to about 12 ppt (Simmonds and Berseth 1977), but was in the lower part of this range much of the early and mid 1970's (Abbe 1977).

Loosanoff (1953) established that oysters do not grow well at salinities less than 7.5 ppt, and feeding ceases below 5 ppt (Galtsoff 1964).

#### MATERIALS AND METHODS

In December 1976, at the request of PEPCO, we began a study of the growth and mortality of three size classes of the oyster *Crassostrea virginica* (Gmelin) in the discharge canal and on the river side of the intake curtain wall of the Morgantown plant (Figure 1). This effort monitored the several million hatchery oysters which had been transferred to the discharge canal from Shady Side, Maryland, by Frank Wilde in November 1976. Size classes were approximately 20 mm (class I), 40 mm (class II), and 80 mm (class III) in shell height (length), and two replicates of 10 of each class were located at the intake (total 60 oysters) and at four sites in the discharge canal (total 240 oysters). In April 1977, a similar set (control) was placed in a tidal

creek in Shady Side near the hatchery oysters which had been removed from the discharge canal and returned to Shady Side for the summer. Except during the winter, oysters were measured approximately once each month.

Two replicates of five additional 70- to 90-mm oysters were also collected monthly from each of the three areas for determination of copper and nickel concentrations. These oysters were scrubbed, rinsed with distilled water, shucked, rinsed again, and blotted dry. The five oysters of each replicate were then pooled and homogenized. A 5-g sample of each replicate was weighed, digested with concentrated nitric acid, and analyzed with a Perkin-Elmer 460 atomic absorption spectrophotometer equipped with a HGA 2100 graphite furnace. Metal concentrations are reported in mg/kg wet tissue weight.

In April 1977 and May 1978, when the overwintered hatchery oysters were returned to Shady Side, approximately 120 of the discharge canal oysters used for metal

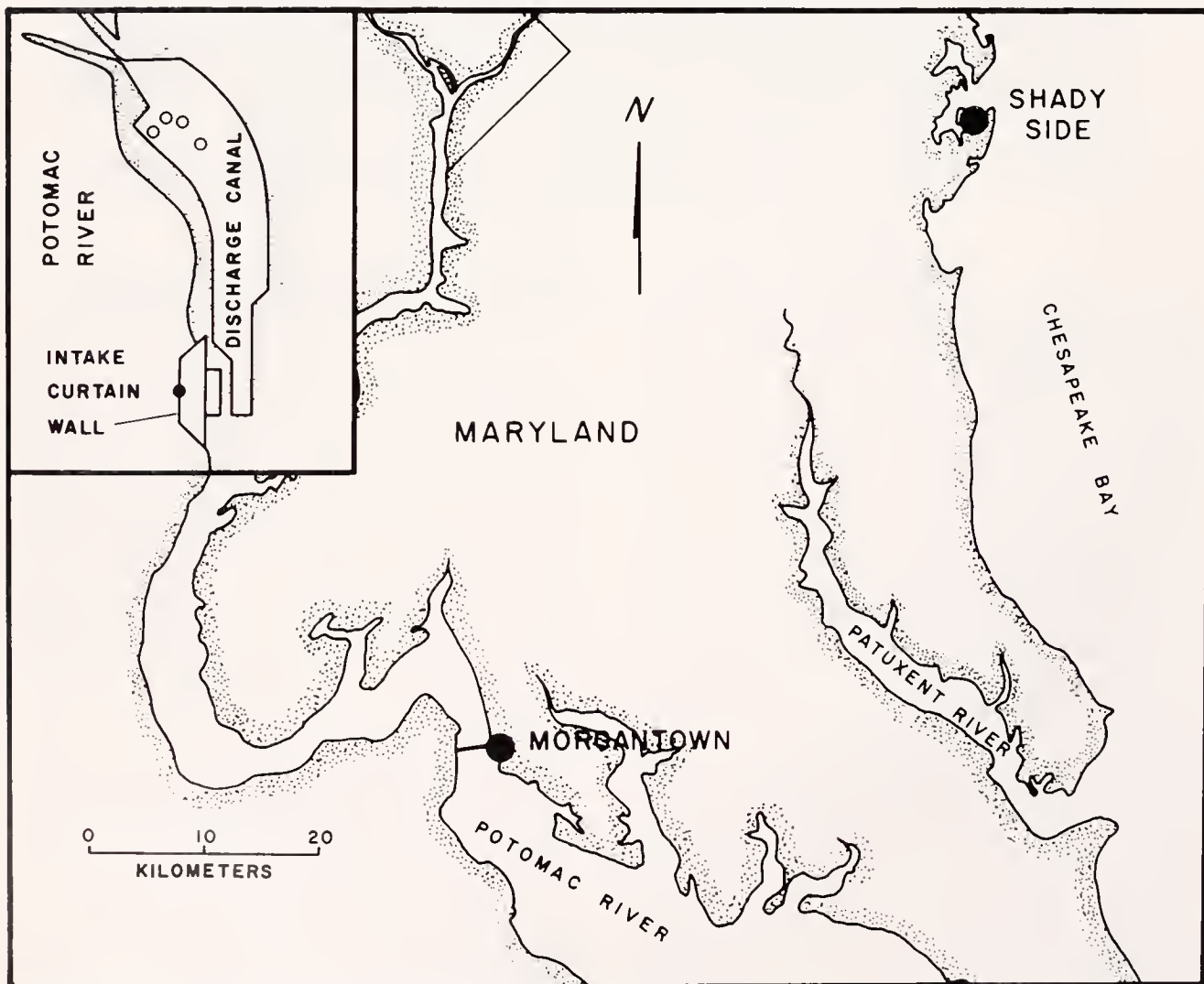


Figure 1. Location of study area showing experimental site at Morgantown and control site at Shady Side, Maryland. The inset shows the locations of oysters at the intake curtain wall (solid circle) and in the discharge canal (open circles) of the Morgantown generating station.

concentration analysis were also returned to Shady Side. These oysters were held in trays with the growth-study control oysters, and allowed the determination of changes in copper and nickel concentrations.

To observe the growth of small oysters during the second year, two replicates of 50 31-mm oysters (class 0) were set out in October 1977 at Shady Side, in the discharge canal, and on the embayment side of the intake curtain wall. These were measured along with the other three classes until November 1978.

Temperature and salinity were determined with a Beckman RS5-3 portable salinometer when oysters were measured. Additional temperature and salinity data were obtained for Shady Side from hatchery records provided by Frank Wilde, and additional temperature data were obtained for the Morgantown station from PEPCO operating records. Morgantown salinity was measured only at the time of oyster measurements.

#### Statistical Analyses.

Oyster growth (length) data were analyzed using regression techniques (Sokal and Rohlf 1969) to compare growth curves of oysters at different stations. A growth curve was derived for each size class of oysters at each station (Jackson and Douglas 1979), and comparisons were made among stations for each size class after initial sizes were adjusted to equal those of controls. All stations were compared simultaneously to determine whether any station differences existed. When a difference was detected, all possible pairwise comparisons were made using the regression procedure described below.

The model used to describe growth in this analysis was:

$$y = \alpha x^{\beta} e^{\epsilon}$$

where  $y$  = length in millimeters,  
 $x$  = age in days,  
 $e^{\epsilon}$  = random error assumed to have log-normal distribution, and  
 $\alpha, \beta$  = parameters to be estimated.

This model was chosen rather than an exponential growth model such as the von Bertalanffy or Krüger growth equations to facilitate hypothesis testing with linear statistical models.

This model was transformed by logarithms to yield a model tractable for linear regression methods:

$$\ln(y) = \ln(\alpha) + \beta \ln(x) + \epsilon$$

The statistic used to test whether growth curves of a group of stations could be satisfactorily explained by a single equation was:

$$F_{v_1, v_2} = [(SSE_p - SSE_g)/v_1] / [SSE_g/v_2]$$

where  $SSE_p$  = error sum of squares from a regression with all stations included,  
 $SSE_g$  = sum of error sums of squares from regressions fitted to stations separately,  
 $v_1$  = reduction in error degrees of freedom by doing separate regressions rather than a pooled regression, and  
 $v_2$  = sum of the error degrees of freedom from the separate station regressions.

Mortality data were analyzed using restricted Chi-square tests with rows as stations and columns as live-dead.

#### RESULTS

Mean monthly temperatures ranged from near 0° to 27° C at Shady Side, from 1° to 29° C at the intake, and from 7° to 35° C in the discharge canal (Figure 2). Mean discharge-canal temperature was about 6° above mean intake temperature. These temperatures are means, however, and although the discharge averaged 7° in January 1977, it fell to 2° following a temporary plant shutdown, well below the 6 to 8° C generally required for oysters to feed (Galtsoff 1964). Shady Side temperatures generally rose more slowly in the spring and fell more rapidly in the fall than did the intake temperatures, but the difference between them was much less in 1978 than in 1977 (Figure 2).

Salinity at Morgantown was 5.0 to 12.4 ppt during 1977 and 1.5 to 12.6 ppt during 1978 (Figure 3). These ranges appear similar, but the patterns were very different. In 1977, the annual mean salinity was 9.1 ppt (no January data). Only during March, April, and May was salinity below 7.5 ppt, and at no time during the year did it fall below 5.0 ppt. In contrast, the 1978 annual mean salinity was 5.6 ppt (no December data), and from January to July the monthly means were less than 5.0 ppt.

Salinity at Shady Side averaged 8.5 ppt during 1977, and as at Morgantown there were only 3 months (February–April) when salinity was below 7.5 ppt. However, the 1978 pattern was quite different from that which occurred at Morgantown. The annual mean was 9.2 ppt, and of the 4 months in which salinities were below 7.5 ppt, only in May was it less than 5.0 ppt.

#### Growth.

Growth of the oysters at the three locations also contrasted between the two years. During 1977, oysters grew well from June to October (Figure 4), but in 1978 growth was poor until August. Through May 1977, oysters at Morgantown showed very little increase in size, while controls showed substantial increases from late April to the end of May. The suspected reason for this difference, a 5.8 ppt salinity at Morgantown and 8.0 ppt at Shady Side, is seen in Figure 3. Once salinity was above 7.5 ppt in both areas, growth rates were similar until October. During October 1977, the growth of both intake and control

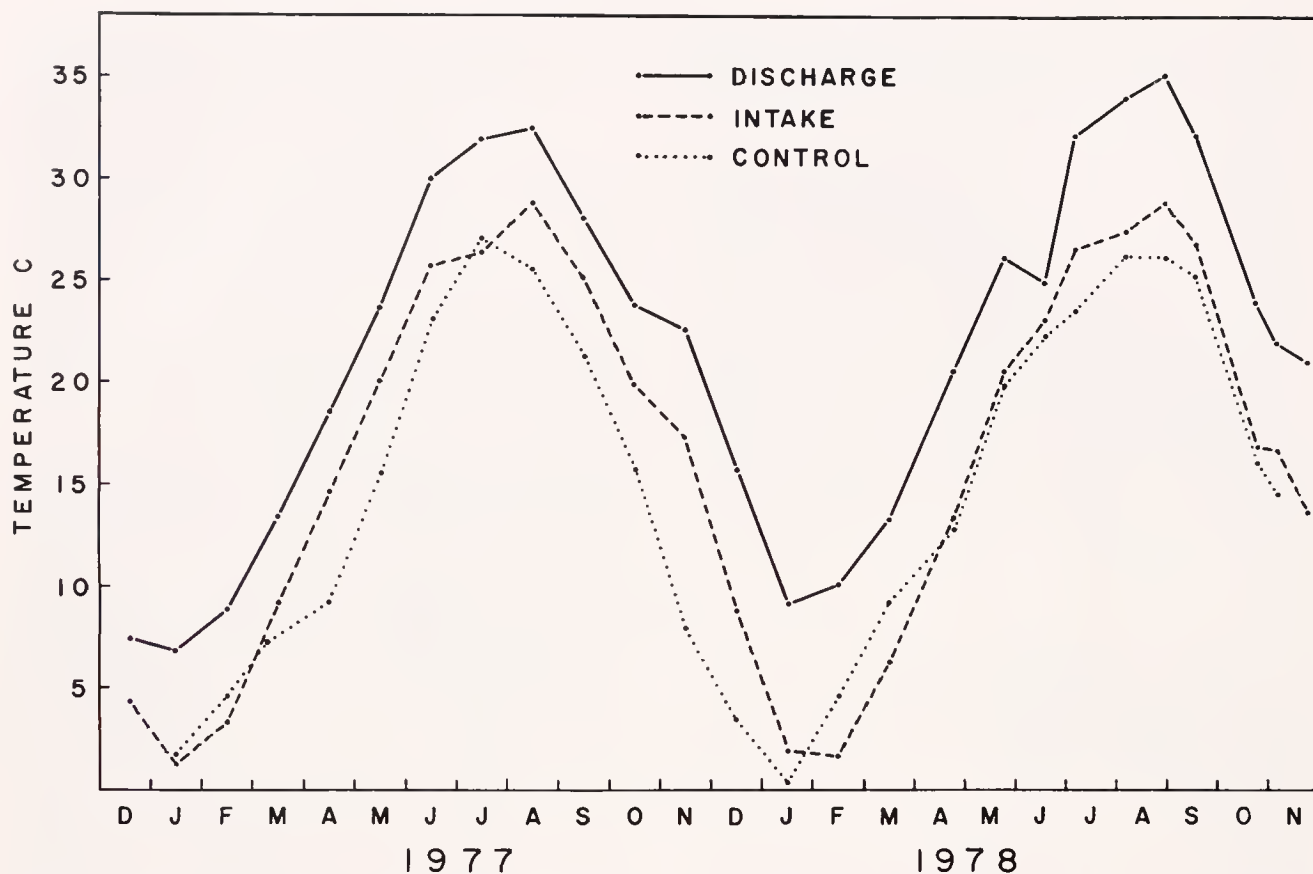


Figure 2. Monthly temperatures in the intake and discharge canal of the Morgantown Steam Electric Station and in Chesapeake Bay at Shady Side, Maryland, from December 1976 to November 1978.

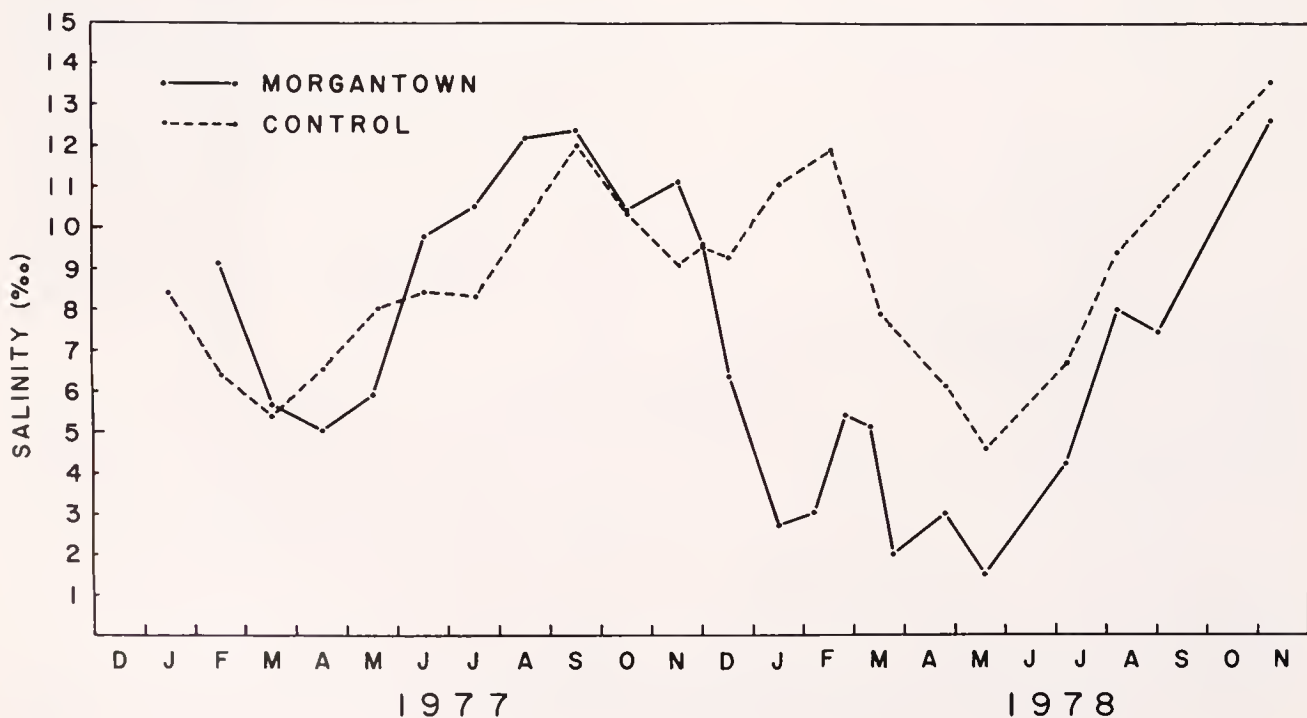


Figure 3. Monthly salinities at Morgantown and Shady Side, Maryland, from December 1976 to November 1978.

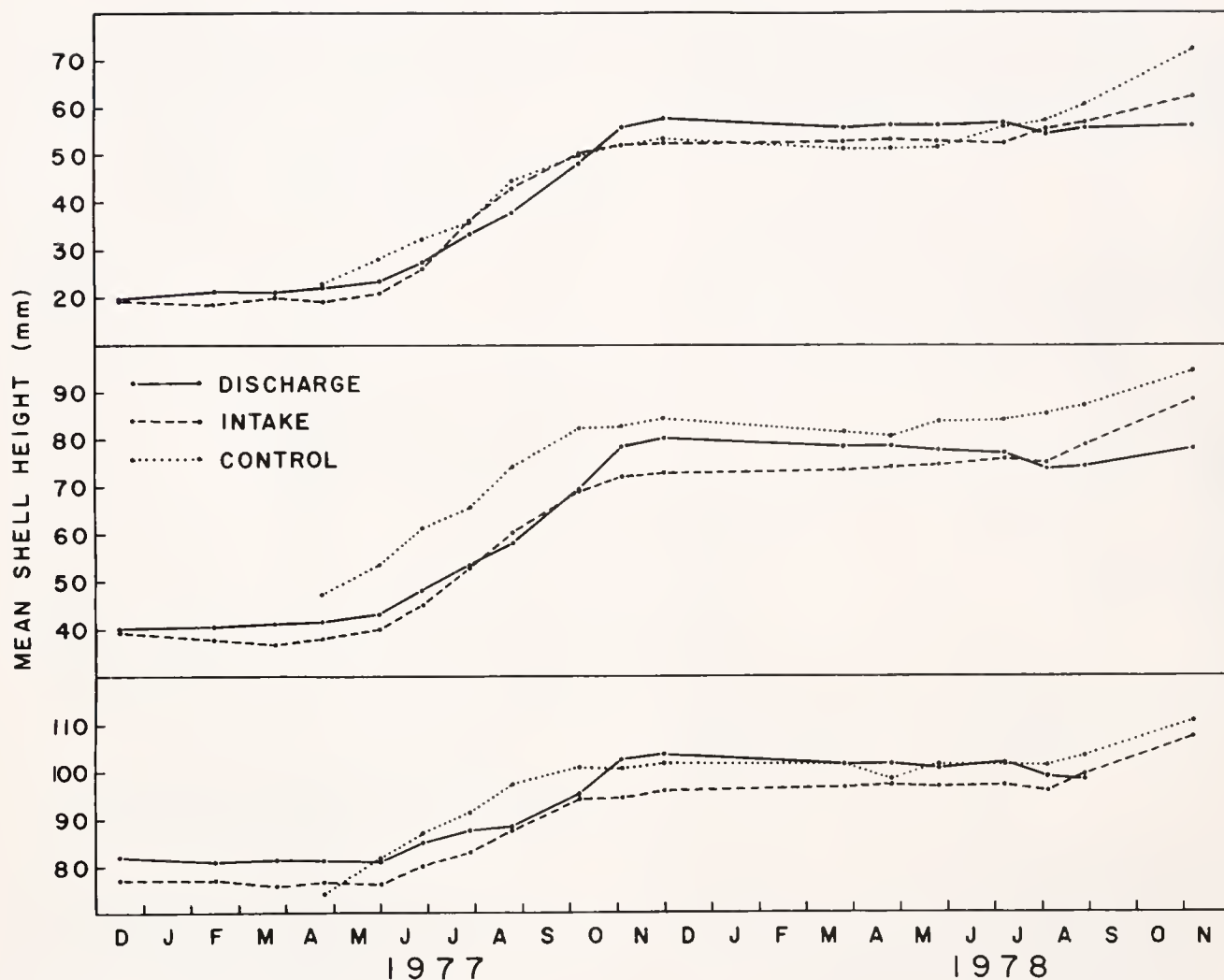


Figure 4. Growth of three size classes of intake, discharge-canal, and control oysters during 1977–1978. (Class I is at top; class III is at bottom.)

oysters slowed considerably while those in the discharge canal showed increased growth (Figure 4).

From December 1977 through April 1978, growth of all oysters was negligible. In fact some decrease in size was observed, a common occurrence in this area due to normal winter attrition of oyster bills. By May, control oysters began to show slight size increases. Salinity was only 4.6 ppt at Shady Side, but it increased rapidly (Figure 3). At the intake, growth did not begin until July when salinity finally approached 5.0 ppt. From August until November, as salinities in both areas increased to above 12.0 ppt, growth of intake oysters was similar to that of control oysters. Discharge-canal oysters showed no net increase in size during 1978; the size decreases from December 1977 through July 1978 were not offset by the slight gains made from August to November 1978. The class III discharge-canal oysters were not included in the growth analysis after August 1978 because all but three had died.

The class 0 oysters showed a growth pattern similar to

that observed for the other classes. From October to December 1977, those in the discharge canal outgrew both intake and control oysters, probably because of higher discharge-canal temperatures which were still 16°C in December compared to 9° at the intake, and only 3° at Shady Side (Figure 2). Galtsoff (1964) states that feeding ceases at 6 to 7°C, but Shaw (1962) saw no growth of rafted oysters in Massachusetts when the temperature was below 10°. Obviously, the metabolic activity of discharge-canal oysters was much greater than that of oysters from the other two areas during this period. Following the October–December growth period and proceeding through the end of August 1978, the discharge oysters began a slow decrease in mean size (Figure 5). A size increase was observed from August to November, but by then nearly all were dead. Intake oysters began to grow in June 1978 (Figure 5), when the salinity was still well below 5.0 ppt. This was both unexpected and unexplainable. The control oysters, after decreasing in size from December to March,

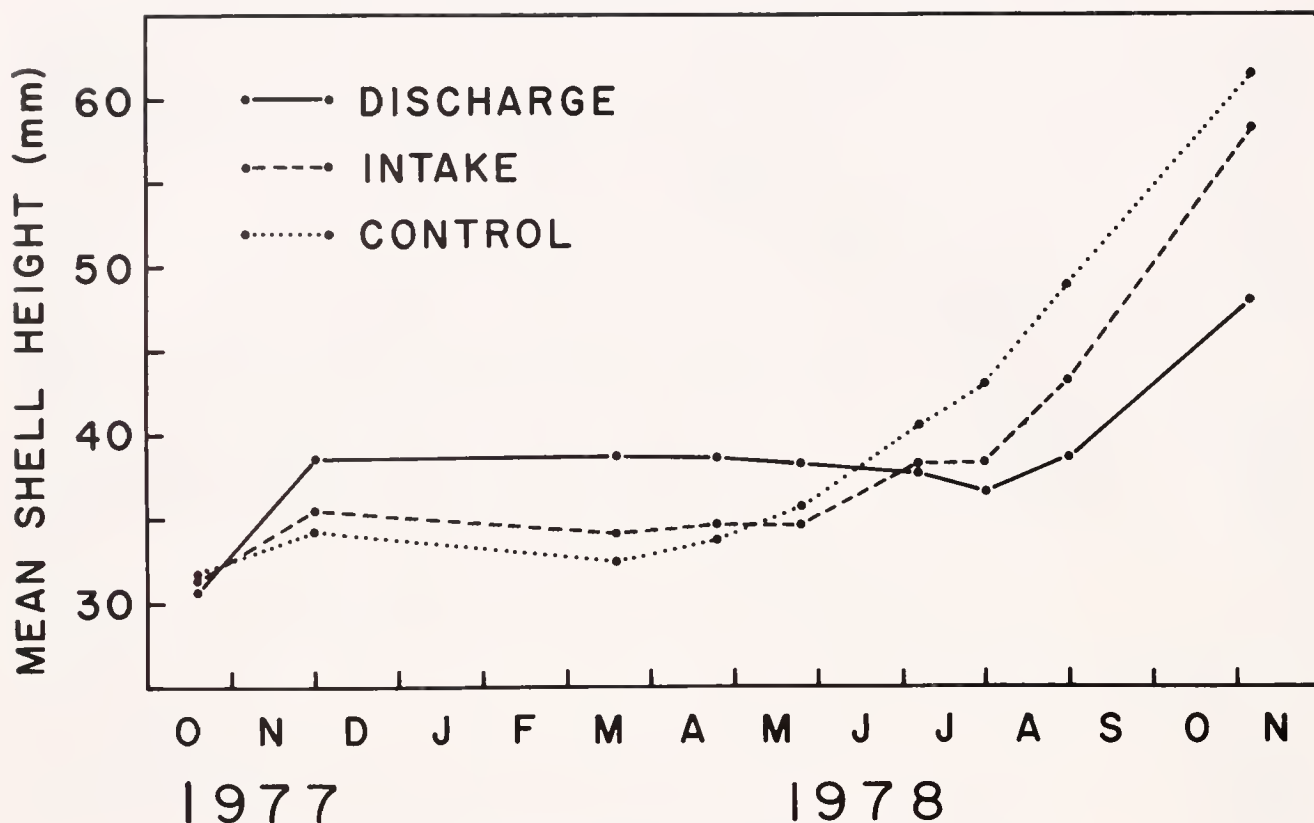


Figure 5. Growth of intake, discharge, and control class 0 oysters during 1977–1978.

began steady growth which continued until November. Growth rates for intake and control oysters were similar from June to November despite the  $\approx 2$  ppt lower salinity in the Morgantown area during the period.

Data analyses revealed significant differences in growth among stations for all size classes (Table 1). Pairwise comparisons indicated that growth of controls in all four classes was significantly greater than in the discharge canal ( $p < 0.001$ ). Growth of intake oysters was also significantly greater ( $p \leq 0.002$ ) than that of discharge oysters in three of four classes (class III,  $p = 0.62$ ). Class 0 and III controls grew more than intake oysters ( $p < 0.001$ ), but class II intake oysters outgrew controls ( $p = 0.002$ )(class I,  $p = 0.56$ ).

#### Mortality.

Cumulative mortality in all classes ranged from 0 to 32% after 1 year (Table 2), and from 0 to 96% after nearly 2 years (0 to 97% after 1 year for class 0 oysters). A comparison of oyster mortalities among stations and the significance levels associated with differences are shown in Table 3. During the first year, class I oyster mortality was significantly higher in the discharge canal than in the other areas, and during the second year this difference was detected for all classes (all  $p < 0.01$ ). Also during the second year, class 0 and class I intake oysters showed higher mortality than controls ( $p < 0.05$ ).

TABLE 1.

Comparison of shell growth of four size classes of oysters in the Morgantown plant intake (IN) and discharge canal (DC) and at Shady Side (SS).

	F ratio	Significance
Class 0		
IN > DC	30.42	<0.001
SS > IN	23.59	<0.001
SS > DC	95.47	<0.001
Class I		
IN > DC	13.83	<0.001
SS = IN	0.58	0.560
SS > DC	21.60	<0.001
Class II		
IN > DC	6.18	0.002
IN > SS	6.48	0.002
SS > DC	24.31	<0.001
Class III		
IN = DC	0.48	0.620
SS > IN	40.88	<0.001
SS > DC	51.71	<0.001

Mortalities were not the result of predation, and although no pathological studies were conducted, certain diseases such as Dermo and MSX can be ruled out because of low salinity (Andrews 1964, Sindermann 1970). Bacterial infections,

TABLE 2.

Cumulative percent mortality of oysters held in the intake (IN), discharge canal (DC), and at Shady Side (SS) from December 1976 to November 1978.

Date	Class 0			Class I			Class II			Class III		
	IN	DC	SS	IN	DC	SS	IN	DC	SS	IN	DC	SS
15 Dec 76				—	—		—	—		—	—	
15 Feb 77				0	0		0	0		0	0	
24 Mar 77				0	0		0	0		0	0	
25 Apr 77				5	0	—	0	0	—	0	2	—
31 May 77				5	5	0	0	0	0	0	2	0
27 Jun 77				5	5	0	0	0	0	5	2	0
28 Jul 77				5	15	0	5	4	0	11	4	0
26 Aug 77				5	18	0	5	8	0	11	4	0
4 Oct 77				5	24	0	5	9	0	11	4	0
2 Nov 77	—	—	—	5	30	0	5	9	0	11	4	0
1 Dec 77	0	0	0	10	32	0	5	9	0	11	4	0
21 Mar 78	0	1	0	10	32	0	5	14	11	11	6	11
24 Apr 78	0	1	0	10	32	0	5	14	16	11	6	11
23 May 78	0	1	0	10	32	0	11	14	16	11	6	11
7 Jul 78	2	6	0	15	47	0	11	27	16	25	24	29
1 Aug 78	2	11	0	15	65	0	11	78	21	25	73	29
28 Aug 78	3	26	0	20	76	0	11	92	21	25	89	29
7 Nov 78	5	97	0	20	94	0	17	96	21	25	89	35

TABLE 3.

Comparison of oyster mortality in the intake (IN), discharge canal (DC) and at Shady Side (SS) after 1 and 2 years, and the associated significance levels based on restricted Chi-square tests.

	Class 0	Class I	Class II	Class III
Dec 1977	—	DC > IN, SS (0.01)	NS	NS
Nov 1978	DC > IN, SS (0.01) IN > SS (0.05)	DC > IN, SS (0.01) IN > SS (0.05)	DC > IN, SS (0.01)	DC > IN, SS (0.01)

which would have been more active at the higher temperatures in the discharge canal, may have had some effect, but the main cause of the high discharge-canal mortality was thought to be a combination of high temperature and low salinity during 1978. Temperatures of 28° to 34°C and prolonged salinities below 5 ppt have been shown by Abbe and Hart (1974) and Gilmore et al. (1975) to be lethal to oysters. To test this hypothesis, a stepwise multiple-linear regression (Douglas 1979) was performed using logit-transformed (Cox 1970) monthly survival data against the present and previous month's temperatures and the present and previous 4 months' salinities to yield the combination of temperature-salinity that would best explain the mortality. The analysis indicated that mortalities were best explained when temperature and salinity were lagged

1 and 3 months, respectively, to survival data (Table 4). Because high mortality often occurred 1 month after high temperatures were experienced and 3 months after prolonged low salinities began, these findings support the hypothesis that mortality was related to high temperature and low salinity.

TABLE 4.

Significant effects of temperature and salinity on mortality of various size classes of oysters. (Mortality at the intake and Shady Side was low compared to that in the discharge canal and in most cases could not be explained by temperature and salinity variables; such cases are not presented.)

Variable	Intake	Discharge Canal			
	Class 0	Class 0	Class I	Class II	Class III
Temperature (0)†		**			*
Temperature (1)			**	**	**
Salinity (0)				*	*
Salinity (1)					
Salinity (2)					
Salinity (3)	*	**		**	**
Salinity (4)					

† Number of months the variable was lagged to the mortality data.

\* 0.05 level significance.

\*\* 0.01 level significance.

#### Heavy Metals.

Concentrations of nickel in oyster tissue appear to be unrelated to plant operation. Although intake and discharge concentrations averaged 0.45 mg/kg compared with 0.28 mg/kg for controls during 1977, the differences were not consistent as concentrations fluctuated widely (Figure 6). The 1977 pattern was not repeated in 1978, and although 1978 concentrations began to increase earlier than in 1977, they did not reach 1977 levels. Intake oysters averaged 0.25 mg/kg nickel in 1978 (about half of 1977 levels), and discharge oysters averaged 0.41 mg/kg. Concentrations for control, discharge canal, and intake oysters ranged from 0.04 to 0.47, 0.02 to 1.11, and 0.02 to 1.43 mg/kg wet weight, respectively, during the entire study period. Pringle et al. (1968) found a range of nickel in *Crassostrea virginica* collected from Maine to North Carolina of 0.08 to 1.80 mg/kg with a mean of 0.19 mg/kg. Although the range observed by Pringle et al. (1968) was similar to the ranges detected in this study, the station means at Morgantown were higher. They were also higher than the 0.20 mg/kg mean of 100 oysters analyzed during 1977–1979 from a station located 4 km downstream from the discharge of the Chalk Point plant on the Patuxent River (ANSP 1981). Morgantown nickel concentrations were similar, however, to the mean 0.45 mg/kg (range 0.14 to 0.96 mg/kg) detected in 70 tray-held oysters in the discharge area of the Calvert Cliffs Nuclear Power Plant on Chesapeake Bay during 1978–1980

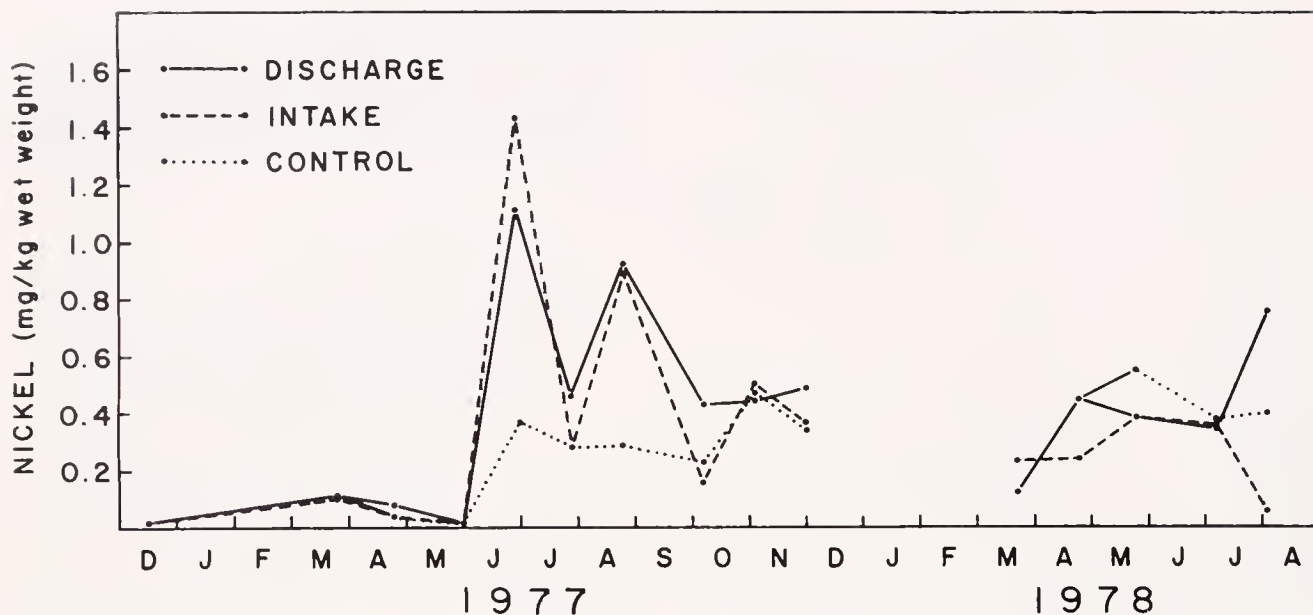


Figure 6. Nickel concentrations determined in intake, discharge, and control oysters during 1977–1978. (Controls were moved from the discharge canal to Shady Side in the spring of 1977 and 1978.)

(Abbe 1981b). Condenser tubes at both Chalk Point and Calvert Cliffs are made of the same copper-nickel alloy as those as Morgantown.

In contrast to nickel, copper concentrations showed a strong relationship to operation of the power plant. The mean concentration among intake oysters increased from 10 mg/kg in December 1976, to 200 mg/kg in May 1977, before declining during June and July (Figure 7). Increases occurred again during August and September. A similar seasonal pattern was observed for discharge oysters, but at much higher levels. They reached 450 mg/kg in May, declined to 270 mg/kg in July, and then increased to 765 mg/kg by October. Intake and discharge-canals oysters averaged 120 and 447 mg/kg, respectively, during 1977. In 1978, copper in discharge oysters remained above 500 mg/kg from March to August, while intake oysters decreased from 132 to 22 mg/kg. Mean concentrations for intake and discharge oysters during 1978 were 100 and 598 mg/kg, respectively. Pringle et al. (1968) found copper concentrations in oysters from Maine to North Carolina to range from 7 to 517 mg/kg wet weight with a mean of 91 mg/kg. As with nickel, the range was similar to that shown by Pringle et al. (1968), but the means were much higher in discharge oysters than in natural populations (Huggett et al. 1973).

These high discharge levels agree with the findings of Roosenburg (1969), although they were much higher than the 1.0 mg/g dry weight (200 mg/kg wet weight) that Roosenburg found. They were also much higher than the 51 mg/kg wet weight reported by Abbe (1981b) for oysters in the discharge area of the Calvert Cliffs plant during 1975–1979.

Within 63 days of transfer to Shady Side in April 1977, copper in discharge oysters decreased from 260 mg/kg to 18 mg/kg (3.84 mg/day) and remained low until the end of the year (Figure 7). In 1978, discharge oysters averaging 510 mg/kg decreased to 258 mg/kg (3.60 mg/day) within 70 days of transfer to Shady Side. During these two periods in 1977 and 1978, oysters remaining in the discharge canal continued to accumulate copper at rates of 1.74 and 2.33 mg/day, respectively. These results indicate higher depletion rates than accumulation rates, even though depletion occurred at lower temperatures (Shady Side versus discharge, Figure 2).

The depuration of copper from 260 to 18 mg/kg, within 2 months after transfer to Shady Side in 1977, was more rapid than that determined by Ikuta (1968) who observed a similar reduction (from 232 to 13 mg/kg in 4 months for *Crassostrea gigas*. Ikuta's study was conducted from August to December as temperatures decreased from 28° to 16°C, whereas the present study indicated more rapid depuration during May and June as temperature increased from 15° to 27°C. Okazaki and Panietz (1981) determined that the biological half-life of copper in *C. virginica* was several times that in *C. gigas*, but those findings were not confirmed by the present study. Perhaps depuration rates are higher as temperature increases through a range than when it decreases through the same range.

## DISCUSSION

Winter discharge-canals temperatures were expected to remain above 10°C with oysters showing continued growth. However, unscheduled plant shutdowns in January 1977 resulted in canal temperatures near 2°C and an end to oyster

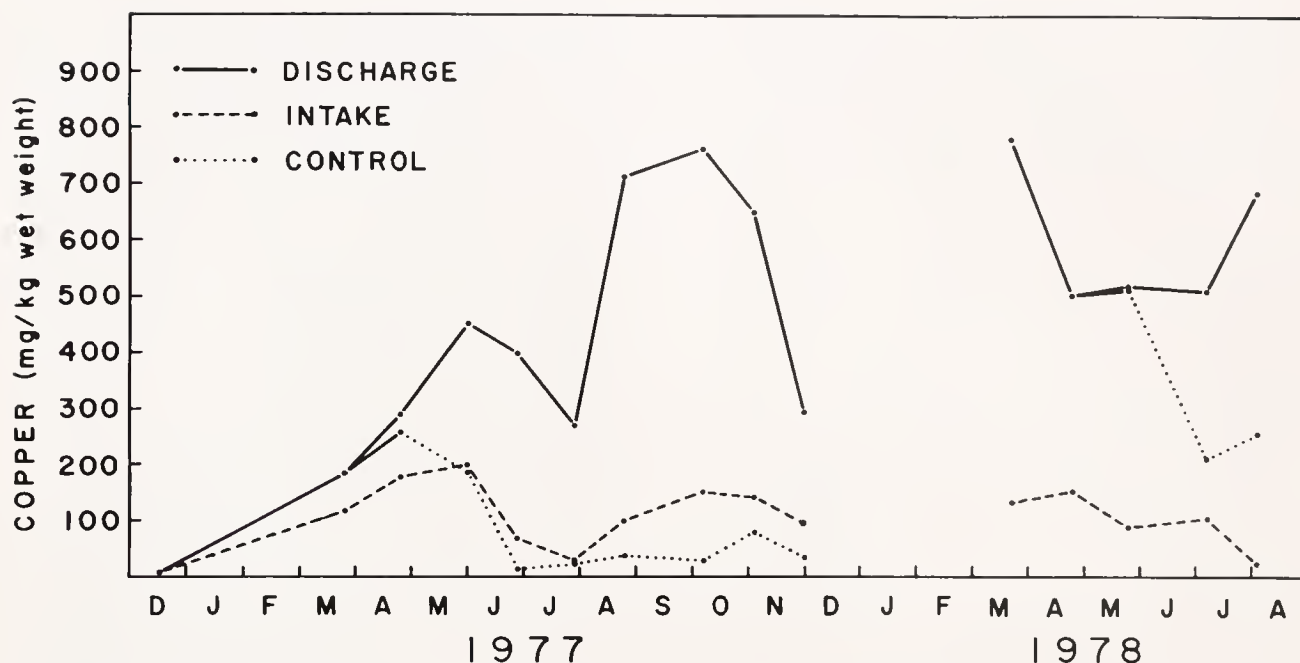


Figure 7. Copper concentrations determined in intake, discharge, and control oysters during 1977–1978. (Controls were moved from the discharge canal to Shady Side in the spring of 1977 and 1978.)

growth until spring. Had the temperature remained above 10°C, some growth may have occurred because salinity was favorable (9.2 ppt in February 1977). During the 1978 winter, canal temperatures remained higher than in 1977 (Figure 2), but by January, salinity had decreased to 3 ppt (Figure 3). Thus, a favorable combination of temperature and salinity during the winter months was never realized.

The fact that oysters in all three areas grew little during 1978 was not unexpected considering that salinity was below 7.5 ppt from April through July at Shady Side and from January through July at Morgantown (Figure 3). Loosanoff (1953) has shown that although oysters will feed at salinities as low as 5 ppt, they grow very little; and growth is also slower at 7.5 ppt than at higher salinities. Higher 1978 mortalities in the discharge canal than at the intake and Shady Side was not surprising either considering they occurred during and following nearly 3 months of temperatures above 32°C. Temperatures of 32 to 34°C impede the normal rate of water transport by the gills and result in reduced respiration, feeding, and growth (Galtsoff 1964). Loosanoff (1953) stated that in fresh and low-salinity water, survival time was controlled by water temperature (the lower the temperature, the longer the survival time). Abbe and Hart (1974) and Gilmore et al. (1975) demonstrated reduced survival time at higher temperatures. Loosanoff (1953) also showed no significant difference in survival of spat and adult oysters in salinities

of 5 ppt or less. Although the smallest oysters in this study cannot be considered spat, the similarity of mortalities among classes of discharge canal oysters as of November 1978, confirms that size has no effect on survival at low salinity. However, the mortality among class 0 discharge oysters was more sudden and complete than among the larger oysters, indicating greater tolerance by small oysters to the combination of high temperature and low salinity during July and August only (Table 2). Whether this tolerance was a function of size, shorter residence time in the discharge, or some other factor is not known.

Although the behavior of the oysters in the discharge canal during 1978 could have been predicted, the 1977 behavior could not have been. The fact that oysters survived and grew about as much as the controls came as a complete surprise. Studies conducted in the Morgantown discharge canal by the Academy of Natural Sciences of Philadelphia in 1971 (Abbe and Krueger 1977a) and in 1976 (Abbe and Krueger 1977b) indicated that the area was not suitable for growing oysters. Oysters held in the canal, primarily for metal analysis, showed little growth, emaciated green meats, and high copper levels, a condition thought to be caused by stresses associated with temperatures elevated several degrees above ambient, salinities which were often 3 to 7 ppt, and low chlorine residuals in an environment enriched with copper from condenser tubes. Except for salinity, these conditions typified the discharge canal during the warm months of 1977; thus, little was expected of

growth and survival. Powell (1973) also concluded that the discharge canal was not suitable for year-round oyster growing because of frequent episodes of low salinity.

Bongers et al. (1977) observed no mortality among oysters exposed to 0.035 to 0.085 mg/l total residual chlorine for 15 days, but reduced shell growth was observed when compared to controls. Although chlorine residuals at the Morgantown condenser outlets (0.02 to 0.05 mg/l) were in the lower end of that range, and although decay continued as the water passes through the canal, low-level residuals were probably present as the water was discharged to the river. In an examination of the effects of chronic exposure of oysters to chlorination, Scott and Middaugh (1978) observed reduced fecal production and condition and gonadal indices during spring at concentrations as low as 0.08 to 0.17 mg/l chlorine-produced oxidant. Although these concentrations are low, they are considerably higher than those experienced by oysters in the Morgantown discharge canal. Maximum shell deposition by juvenile oysters exposed to 0.04 mg/l total residual chlorine by Roberts et al. (1975) was 30% of that of controls; and 50% of control growth at 0.023 mg/l was estimated. Despite probable low-level chlorine residuals, the oysters in the discharge canal appeared unaffected.

Price et al. (1976) stated that oysters in the warmer discharge waters of the Maine Yankee Atomic Power Station showed accelerated growth, but did not mention whether cooling water was chlorinated. Abbe (1981a) has also shown accelerated growth of oysters in discharge waters of the Calvert Cliffs Nuclear Power Plant, but no biocides were used in the cooling water. Tinsman and Maurer (1974) concluded that while oysters were affected by thermal increases from the power plant on Indian River Bay, they were not affected by chlorine because of the high chlorine demand by seawater and the distance from the plant outfall (minimum 2.5 km). These three studies are just a few of many that demonstrate the feasibility of using power plant discharge water to enhance oyster growth, but there is little evidence to show that oysters in chlorinated waters are able to grow as well as controls.

In addition to the possible effect of chlorine on discharge-canal oysters was the uptake of nickel and copper. Because nickel concentrations in oysters from June to October 1977 were similar at the intake and discharge (Figure 6), there appeared to be no direct plant effect. However, the higher copper concentrations in discharge-canal oysters obviously resulted from plant operation (Figure 7). During a 28-week period from March to October 1977, mean copper concentration in discharge oysters increased from 190 mg/kg to 765 mg/kg wet weight, a mean increase of 20.5 mg/kg/wk. Temperature during that time averaged

25°C, but ranged from 13.4° to 32.4°C (Figure 2). These increases were about two thirds of those determined in two separate studies during 1967 (31.2 mg/kg/wk) and 1968 (29.8 mg/kg/wk) by Shuster and Pringle (1969) for oysters exposed to 0.025 ppm copper at 20°C in the laboratory. Nevertheless, as copper concentrations in discharge oysters increased during the season, the oysters continued to grow similar to intake and control oysters (Figure 4). With mean temperatures during June–August ranging from 30° to 32.4°C, high copper concentrations, and probable low-level chlorine residuals, the oysters in the discharge canal grew as though there were no external stresses.

Temperature is clearly an important factor in uptake and depuration of metals, as it controls all metabolic rates (assuming other environmental variables are favorable). Cunningham and Tripp (1973) concluded that mercury accumulation was more rapid than depuration when accumulation studies were done at higher temperatures (July–August) than depuration studies (September–February). Pringle et al. (1968) also showed lower depletion rates for metals at lower temperatures than at higher temperatures. The present study indicated higher depletion rates than accumulation rates, even though depletion occurred at lower temperatures. If temperatures at Shady Side had been as high as discharge temperatures, the difference between depletion and accumulation may have been even greater.

This study confirmed earlier findings (Powell 1973), that winter survival rates in the discharge canal were high. Although oysters are generally not killed by cold water alone (McKenzie [1981] reported no cold-water mortality among oysters as small as 8 mm in Long Island Sound from 1966 to 1972), they can suffer heavy mortality if frozen in shallow water. Therefore, a warm-water area would be more suitable for overwintering oysters than shallow tidal creeks such as those in Shady Side, even though the temperature was not high enough to maintain continuous growth.

The Morgantown discharge canal provided an excellent site to overwinter oysters, and could be used for year-round grow-out if salinity remained high enough. Regardless of the length of time the canal was used, however, oysters would have to be transferred to another area for at least 2 to 3 months to eliminate the accumulated copper.

#### ACKNOWLEDGMENTS

Thanks are due to Frank Wilde for his assistance and the use of his equipment and facilities throughout this study. The efforts of Robert Cantin in the field are deeply appreciated as are those of Elgin Perry for computer analysis of the data. This study was supported by the Potomac Electric Power Company.

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# EPIZOOTIOLOGY OF LATE SUMMER AND FALL INFECTIONS OF OYSTERS BY *HAPLOSPORIDIUM NELSONI*, AND COMPARISON TO ANNUAL LIFE CYCLE OF *HAPLOSPORIDIUM COSTALIS*, A TYPICAL HAPLOSPORIDAN\*

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**ABSTRACT** The two haplosporidan parasites that cause diseases of oysters along the middle North Atlantic coast of North America differ in their habitats, in timing of oyster mortalities, and in their adaptations to the host. *Haplosporidium nelsoni* (MSX) kills oysters throughout the year over a wide range of salinities (about 15 to 30 ppt). It has a long infective period of nearly 6 months. This pathogen rarely completes sporulation in its life cycle in oysters. It is highly pathogenic and exhibits irregular activity suggesting that it is poorly adapted to the host species. In contrast, *Haplosporidium costalis* (SSO) has a short, well-defined mortality period of 4 to 6 weeks; it always sporulates in May-June and promptly kills the host oyster. The infective period is short and initial infections occur during the mortality period. Clinical infections appear after 8 to 10 months of incubation as hidden infections. This pathogen appears to be a native species and exhibits a regular life cycle in the oyster host.

Failure to achieve artificial infections with either pathogen has led most investigators to assume that some unknown host is the primary source of infective particles. Spasmodic attempts to achieve artificial infections are not convincing that direct transmission from oyster to oyster does not occur, given the scarcity of spores of *H. nelsoni* for experiments and the unavailability of *H. costalis* spores to most laboratories.

In Virginia, *H. nelsoni* infections can be divided into early-summer and late-summer acquisitions that result in quite different mortality patterns of oysters. The late-summer infections of *H. nelsoni* exhibit patterns of incubation and mortality similar to those of *H. costalis*. Data are presented for many years of monitoring of late-summer MSX infections and subsequent June-July deaths the following year. The purpose of this report is to call attention to the value of life-cycle studies of *H. costalis* as a typical haplosporidan. The scarcity and dormancy of spores and the often long periods of obscure infections in *H. nelsoni* tend to make infection experiments difficult.

## INTRODUCTION

The sporozoan disease of oysters caused by *Haplosporidium nelsoni* (Haskin et al. 1966) appeared in Chesapeake Bay in the summer of 1959 with severe mortalities in Mobjack Bay and Chesapeake Bay oyster beds. The disease first appeared with oyster mortalities in Delaware Bay in the spring of 1957—just two years earlier (Haskin et al. 1966). It spread rapidly throughout the lower Chesapeake Bay in 1960, wherever summer salinities exceeded 15 ppt (Andrews and Wood 1967). The “Delaware Bay” disease is now endemic in Virginia waters and oysters are no longer planted commercially in high-salinity waters of Chesapeake Bay. The pathogen, also called MSX, has killed oysters in experimental lots regularly every year from 1959 to 1981 (Andrews and Frierman 1974, Andrews 1979a).

Delaware Bay disease has peculiar epizootiological patterns of infection and mortality (Andrews and Frierman 1974). The infective period lasts for 5½ months from mid-

May to 1 November. “Early-summer” infections, those which occur prior to 1 August each year, result in immediate late-summer and fall deaths beginning typically 1 August. “Late-summer” and fall infections remain subclinical for months, and usually are not expressed as mortalities until June-July of the following year. Late-summer infection has not received much attention because its expression as mortalities 7 to 9 months later is obscured by early-summer infections that result in patent infections a month after exposure and deaths beginning 5 to 6 weeks after exposure.

The seasons at which oysters are transplanted from low salinity natural seed areas to high salinity private growing grounds also determine the time of first exposure to *H. nelsoni* (MSX) along the mid-Atlantic coast. In Chesapeake Bay, seed oysters are usually transplanted from 1 October through the winter and spring. Except for the month of October, this is too late for late-summer infection to occur; therefore, most newly transplanted oysters are exposed first to early-summer infection. In Delaware Bay, seed oysters are transplanted to high salinity growing grounds in May and June, hence are exposed immediately to infections. In Virginia, no infections have resulted from monthly importations of disease-free oysters from 1 November to 1 May. The earliest patent infections from May-June

\*Sprague (1978) proposed returning these two species to the genus *Haplosporidium* on the basis of external wrappings of the spores. This gives more generic importance to tails and wrappings than size of spores and site of sporulation. These are fundamental morphological and physiological traits. I question the validity of this generic change.

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exposure occur about 1 July regardless of when experimental oysters are imported between 1 November and 1 June.

The purpose of this report is to point out the parallels in timing and in sequence of events in the life cycles of two haplosporidians, *H. nelsoni* (MSX) and *H. costalis* (SSO). In the comparison, *H. costalis* is considered a far better adapted parasite of oysters. It exhibits a regular annual cycle with mortality and infection periods occurring simultaneously in late spring (May–June). Sporulation occurs completely (all plasmodia become sporonts) and regularly every year. A long incubation period (infections subclinical) permits oysters to reproduce regularly before mortalities occur. In contrast, *H. nelsoni* is highly pathogenic and shows very long infection and mortality periods. It rarely sporulates and does not kill oysters promptly when it does, because only the epithelia of digestive tubules are involved as a site. These irregularities of timing and life-cycle stages make understanding of the epizootiology of *H. nelsoni* confusing. Artificial infections have not been achieved with either parasite. Both parasites occur sympatrically on the seaside of Delmarva peninsula (Couch and Rosenfield 1968).

The epizootiology of late-summer MSX infection appears to have more similarities to that of *H. costalis* than to early-summer infection of *H. nelsoni*. Therefore, data on epizootiological events over a long series of years are presented for comparisons. Late-summer infection of *H. nelsoni* has not occurred in all years; therefore, a comparison of disease success and the environmental factors regulating it may offer clues to life-cycle requirements of the pathogen.

#### METHODS

Sporozoan diseases of oysters were monitored in Virginia for 23 years by importing disease-free lots from low salinity natural beds in the James River seed area. Cohorts of 300 to 500 oysters were imported each spring to 10 stations in three rivers of lower Chesapeake Bay. These stations were chosen to represent a wide range of salinity regimes both within and upriver from the endemic areas for *H. nelsoni*. Only the major monitoring station at Gloucester Point one-half mile above the bridge on the York River was routinely stocked with disease-free oysters in late summer to follow that infection period and its subsequent mortality expressed the following June–July. Data from the Gloucester Point station are the basis for this report.

Tray monitoring permitted choices of oyster stocks as to age, history, and timing of exposure which are not readily feasible or available in commercial plantings. Routine counts every 2 to 4 weeks provided accurate data on mortalities caused by diseases, without interference by predators and smothering. Tray monitoring procedures are those described by Andrews (1966) and Andrews and Frierman (1974). Samples of 25 live oysters were taken at appropriate times to document earliest appearance and

peak prevalences of infections. Monthly and annual mortalities were calculated using Ricker's (1958) table of instantaneous rates when oysters were removed or lost.

Fortunately for monitoring purposes, *H. nelsoni* does not vary widely in activity with local geography providing its salinity requirements are met (Andrews and Frierman 1974). Another pathogen, *Perkinsus marinus*, requires close proximity of oysters to produce new infections, and dense host populations are needed for it to multiply and spread rapidly (Andrews 1979a). Therefore, to monitor *H. nelsoni*, which exhibits no proximity effect, it is possible to isolate trays and beds of oysters from *P. marinus*. Also, large areas with similar or adequate salinities can be monitored for *H. nelsoni* with a few trays of oysters. Density of oysters in trays is not a problem with *H. nelsoni*. Trays of oysters as widely spaced as Gloucester Point in the York River, New Point Comfort in Mobjack Bay, and Hampton Bar in the James River exhibited similar timing and levels of infections and mortalities each year (Andrews and Wood 1967).

The greatest variations in *H. nelsoni* activity were caused by the degree of susceptibility of various oyster strains to MSX and the amount of previous selection by the disease. It is important to describe the source and history of oyster stocks used for monitoring (Haskin and Ford 1979, 1982). From 1960 to 1980, experimental oysters were obtained from the lowest salinity upriver seed beds in the James River (Horsehead Rock and Deep Water Shoals). Oysters from these two areas are the most susceptible to MSX infection of any in the James River. When oysters from downriver beds were used, as a result of scarcity upriver, observed prevalences and mortalities were lower. Because spatfalls throughout the seed area came from the same larval swarms with the same set of genomes (Andrews 1979a, 1982), exposure to MSX and selection by mortality must have reduced the susceptibility of the lower river stocks. Oysters from upriver beds of the Potomac River were far more susceptible than any stocks from the James River (Andrews 1968). No significant change in susceptibility to MSX in upriver stocks can be detected over the 23 years despite some genetic selection of broodstocks in the lower river. Native oysters in the areas endemic for MSX were slower to develop infections and exhibited lower mortalities than control lots from upriver beds.

The MSX activity for a given year was rated as light, moderate or average, and heavy based upon observed levels of infection (prevalences) and mortalities. For late summer infection, peak prevalences occurred the following May or early June just before mortalities began. The average May prevalence, excluding years in which no infection occurred, was about 50%. Mortalities from late summer infections averaged 40% and were confined mostly to June and July. First appearance of infections and occurrence of mortalities were early in heavy and late in light MSX years (Andrews and Frierman 1974).

## RESULTS

*Patterns of H. nelsoni Activity in Oysters First Exposed in Late Summer and Fall*

Prevalences of MSX and ensuing mortalities in Horsehead Rock (James River) oysters imported to the Gloucester Point station (York River mile 06) in late-summer and fall of 1979 are shown in Figure 1. Infections appeared moderately early in tray Y106 but were about average in Y107. Samples were not taken early or late enough to determine earliest appearance of MSX or peak levels of infections. Mortalities were confined mostly to June and July and totaled 43% for the first pair of peaks in Figure 1. A rapid decline in the death rates in late July shows the near end of mortality from late-summer infection. The delay in timing of prevalences and mortalities in tray Y107, which was imported 7 weeks later than Y106, does not always occur from late-summer exposures (Andrews 1966).

Mortalities from early-summer 1980 exposure to MSX began 1 August, at the typical time. The total losses in this second pair of peaks varied more than usual, but showed typical timing in the decline of death rates in the fall. Death rates are plotted at the end of periods of observation, so that the points in November, for example, represent what happened during the first 20 days of November. Total mortalities from late-summer 1979 and early-summer 1980 infections were 63% and 70%, respectively. After over 20 years of monitoring MSX, intensive sampling of live

oysters was not necessary for interpretation of mortality graphs. In the controlled situation of tray culture, where size, source, and history of oysters are known, a few gapers and limited live-oyster samples at known stages in the life cycle will suffice to determine MSX as the cause of mortalities (Andrews 1966, 1968, 1979a; Andrews and Frierman 1974).

*Compilation by Years of Peak MSX Prevalences and Mortality Rates in Susceptible Oysters over Two Decades*

Peak prevalences in May-June and resultant mortalities in June-July were observed for 14 years after late-summer exposures (Table 1). A high proportion of those deaths was associated with MSX infections as shown by diagnoses of the disease in gapers (Table 2). High prevalences were associated with high mortalities and years of low MSX infections exhibited low mortalities. Most deaths were confined to June and July; therefore, that period of mortality was used for comparison with May-June prevalences from late-summer infection. Variations from year to year in timing of infections and deaths do not permit refined statistical analysis of MSX infections versus mortalities, although the relationship is obvious in a broad sense (Table 1 footnotes). These variables are discussed later. No other disease or cause of death has been identified in oysters imported in late summer.

Prevalences vary far more widely than mortalities (Table 1). This is largely a consequence of the times of

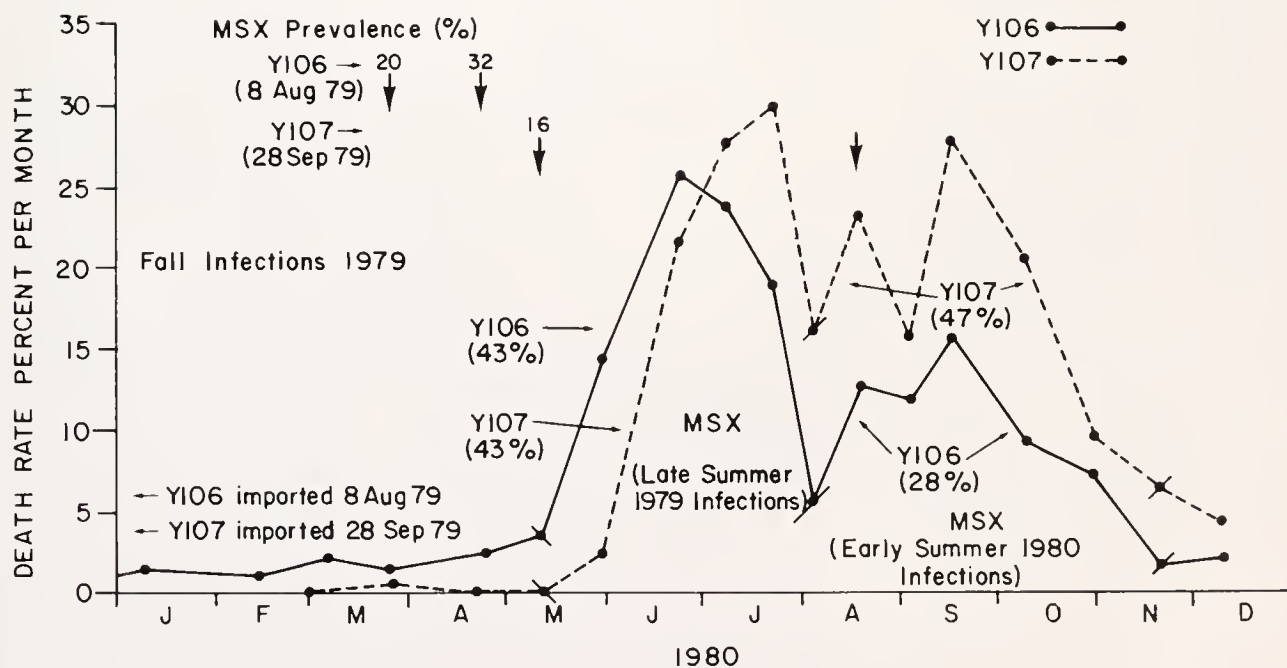


Figure 1. This graph illustrates typical mortalities from late summer transplanting of susceptible oysters from the low salinity James River seed area (Horsehead Rock oysters) to higher salinity areas where *H. nelsoni* is endemic. Exposure in late summer 1979 resulted in deaths from MSX in June and July 1980. New infections in early summer 1980 (prior to 1 August) caused additional mortality in late summer and fall. Prevalences of MSX in lots of 25 live oysters are shown above arrows designating sampling dates. Tray monitoring of oysters for Delaware Bay disease was done at Gloucester Point, VA, 0.8 km (0.5 m) above the York River bridge (mile 06 in York River).

TABLE 1.

Summary of late-summer infection of *Haplosporidium nelsoni* (MSX) in York River, VA, at Gloucester Point, 1963–1979.  
May–June prevalences of disease and June–July mortalities in susceptible oysters from James River.

Tray	Date of Importation	Earliest MSX Infections		Peak of MSX Infections		June–July Mortalities (%)
		Date	Prevalences (%)	Date	Prevalences (%)	
Y18	13 August 1963	8 April 1964	16	25 May 1964	56	29
		18 February 1965	76	3 June 1965	44	
Y21	11 September 1964	18 February 1965	48	12 May 1965	68	41
				3 June 1965	56	
Y25	16 August 1965	15 December 1965	24	11 May 1966	76	40
				29 June 1966	56	
Y34	17 August 1966	14 December 1966	64	6 April 1967	80	62 <sup>a</sup>
		20 February 1967	60	29 May 1967	80	
Y35	17 August 1966	20 October 1966	28	6 April 1967	64	52 <sup>b</sup>
		14 December 1966	80	29 June 1967	40	
		16 February 1967	64			
Y43	21 August 1967	1 December 1967	4	13 May 1968	24	39
		21 March 1968	4			
Y44 (PR)	6 September 1967	1 December 1967	8	13 May 1968	20	35
		30 January 1968	0	9 June 1969	64	
Y45 (PR)	6 September 1967	28 March 1968	0	13 May 1968	28	43
Y46	13 September 1967	1 December 1967	0	13 May 1968	4	29
		30 January 1968	0			
Y54	29 August 1968	failure of infection		1 May 1969	0	5
				16 June 1969	0	
Y72	20 September 1971	22 May 1972	16	22 May 1972	16	4
Y79 (WS)	29 August 1973	31 May 1974	4	31 May 1974	4	14
Y82 (WS)	29 August 1974	21 February 1975	60	19 May 1975	64	44
		21 March 1975	52			
Y86 (WS)	26 August 1975	---	---	---	---	22
Y90	1 September 1976	---	---	18 May 1977	72	40 <sup>c</sup>
Y91	24 September 1976	---	---	18 May 1977	64	54
Y95 (RR)	17 August 1977	---	---	---	---	16
Y96 (RR)	12 October 1977	---	---	30 May 1978	31	15
Y101 (RR)	28 August 1978	9 March 1979	8	25 April 1979	20	29
Y106	6 August 1979	26 March 1980	20	24 April 1980	32	42 <sup>d</sup>
Y107	26 September 1979	12 May 1980	16	12 May 1980	16	39

PR = Potomac River; WS = Wreck Shoal, James River; RR = Rainbow Rock, James River.

<sup>a</sup>+16% mortality before 1 June 1967 (29 of 33 gapers with MSX).

<sup>b</sup>+16% mortality before 1 June 1967 (14 of 17 gapers with MSX).

<sup>c</sup>+9% mortality before 1 June 1977 from MSX.

<sup>d</sup>+9% mortality before 12 May 1980.

TABLE 2.

Chronological prevalences of MSX in susceptible James River oysters imported to Gloucester Point in late summer and held in trays (samples of 25 live oysters).

Tray <sup>1</sup>	Date of Importation	Dates Sampled*	MSX Activity		Tray <sup>1</sup>	Date of Importation	Dates Sampled*	MSX Activity	
			No. of Infections	Intensity <sup>2</sup> (H,M,L,R)				No. of Infections	Intensity <sup>2</sup> (H,M,L,R)
Y18	13 Aug 63	25 Oct 63	1	0-0-1-0			22 Jul 69	5	1-1-2-1
		18 Feb 65	19	5-2-11-1			10 Mar 70	8	1-0-6-1
		3 Jun 65	11	3-1-6-1			18 May 70	0	---
		20 Oct 65	3	1-0-2-0			1 Jun 70	3	0-0-3-0
		Gapers 12	8	1-1-6-0			Gapers 0	---	---
Y21	11 Sep 64	27 Jan 65	6	0-2-2-2	Y65(WS)	2 Sep 79	6 Nov 70	3	0-0-2-1
		18 Feb 65	12	0-0-12-0			5 Feb 71	2	1-1-0-0
		12 May 65	17	2-0-15-0			14 May 71	2	0-1-1-0
		3 Jun 65	14	7-0-6-1			14 Jun 71	0	---
		18 Oct 65	7	1-1-3-2			14 Jul 71	1	0-0-1-0
		Gapers 19	13	2-5-5-1			10 Aug 70	2	0-0-2-0
Y25	16 Aug 65	15 Dec 65	6	0-2-3-1			12 Oct 71	3	0-2-1-0
		11 May 66	19	0-3-14-2			7 Dec 71	3	1-0-2-0
		29 Jun 66	13	4-2-6-1			Gapers 3	2	0-1-0-1
		23 Aug 66	15	1-1-12-1	Y72	20 Sep 71	2 Feb 72	0	---
		23 Jan 67	11	5-1-4-1			22 May 72	4	3-0-1-0
		Gapers 68	48	21-2-23-2			7 Aug 72	3	0-0-2-1
Y34	17 Aug 66	14 Dec 66	16	8-3-5-0			Gapers 0	---	---
		20 Feb 67	15	6-2-4-3	Y79(WS)	29 Aug 73	26 Nov 73	2	2-0-0-0
		6 Apr 67	20	12-1-5-2			31 May 74	1	0-0-1-0
		29 May 67	20	12-3-5-0			2 Aug 74	6	2-0-3-1
		Gapers 33	29	22-1-6-0			Gapers 13	8	2-5-1-0
Y35	17 Aug 66	20 Oct 66	7	3-2-1-1	Y82(WS)	29 Aug 74	11 Sep 74	2	1-0-1-0
		14 Dec 66	20	3-15-1-1			21 Feb 75	15	7-0-8-0
		16 Feb 67	16	10-0-5-1			21 Mar 75	13	7-1-3-2
		6 Apr 67	16	4-3-9-0			19 May 75	16	14-1-1-0
		29 Jun 67	10	5-1-3-1			Gapers 5	4	2-2-0-0
		Gapers 17	14	8-0-5-1	Y85	26 Aug 75	6 Apr 76	5	1-0-2-2
Y43	21 Aug 67	9 Oct 67	0	---			13 May 76	8	7-0-1-0
		1 Dec 67	1	1-0-0-0	Y86(WS)	26 Aug 75	13 Jan 76	1	1-0-0-0
		30 Jan 68	2	1-0-1-0			10 Aug 76	5	1-0-3-1
		21 Mar 68	1	0-0-1-0			Gapers 6	5	2-2-1-0
		13 May 68	6	0-0-6-0	Y90	1 Sep 76	1 Sep 76	0	---
		31 Oct 68	6	1-0-5-0			18 May 77	18	5-6-7-0
		1 May 69	4	0-2-1-1		(17 oysters)	22 Jun 77	8	1-6-1-0
		Gapers 0	---	---			29 Jul 77	6	2-2-1-1
Y44	6 Sep 67	1 Dec 67	2	0-2-0-0			29 Oct 76	1	0-1-0-0
		30 Jan 68	0	---			Gapers 25	14	1-7-3-3
		28 Mar 68	0	---	Y91	24 Sep 76	18 May 77	16	5-6-5-0
		13 May 68	5	0-0-5-0			Gapers 3	1	0-1-0-0
		7 Aug 68	9	2-1-5-1	Y95	17 Aug 77	26 Sep 77	3	0-1-2-0
		1 May 69	6	3-1-1-1			6 Oct 77	0	---
		9 Jun 69	16	9-1-4-2			26 Mar 81	4	0-0-1-3
		Gapers 8	8	2-3-3-0	Y96	12 Oct 77	Gapers 40	20	7-7-5-1
Y45(PR)	6 Sep 67	28 Mar 68	0	---			Gapers 25	20	6-8-5-1
		13 May 68	7	0-0-5-2	Y101(RR)	28 Aug 78	31 Aug 78	0	---
		4 Nov 68	8	2-3-3-0			9 Mar 79	2	0-0-1-1
		Gapers 35	30	11-5-10-4			24 Apr 79	5	0-2-3-0
Y46	13 Sep 67	1 Dec 67	0	---			2 Aug 79	1	0-1-0-0
		30 Jan 68	0	---	Y106	6 Aug 79	Gapers 25	16	4-8-4-0
		28 Mar 68	0	---			26 Mar 80	5	1-1-0-3
		13 May 68	1	0-1-0-0			24 Apr 80	8	1-0-4-3
		7 Aug 68	7	4-0-2-1	Y107	26 Sep 79	Gapers 31	30	18-10-2-0
		27 Sep 68	8	0-1-7-0			12 May 80	4	1-1-1-1
		Gapers 0	---	---			Gapers 7	7	7-0-0-0
Y54	29 Aug 68	20 Dec 68	0	---					
		14 Mar 69	0	---					
		1 May 69	0	---					
		16 Jun 69	0	---					

PR = Potomac River; WS = Wreck Shoal, James River; RR = Rainbow Rock, James River.

<sup>1</sup>All lots from Horsehead Rock or Deep Water Shoals unless otherwise designated.

<sup>2</sup>Intensities of infections: heavy (H), moderate (M), light (L), and rare (R), based on number of plasmodia per 100X field of >5, 1-5, <1, and hard to find or very localized, respectively.

\*All gapers collected in calendar year after importation and exposure to show association with MSX.

sampling, for development of the disease is rapid in late spring. With MSX appearing as early as October in high-prevalence years and as late as 1 June in low-kill years, sampling was often inadequate to disclose a full picture of development of the disease. Samples taken too early or too late failed to reveal peak prevalences, and oyster deaths beginning in June, decreased prevalences. After 1 July, when new infections from early-summer exposure were likely to appear, prevalences could not be definitely related solely to late-summer exposure.

#### *Seasonal Development and Intensity of Late-Summer Infection*

The seasonal progression of clinical MSX infections in late summer and fall importations of disease-free James River oysters is shown in Table 2. In the early years (1960–1963) of light MSX activity in Virginia waters, the pathogen remained nonclinical until May of the following year and prevalences were about 50% in May. Data for 1961 and 1962 were given by Andrews (1966). In the next 3 years (1964–1966), systemic infections appeared in late fall or early winter and prevalences as high as 80% were found in live oysters. Duplicate trays Y34 and Y35, imported 17 August 1966, depict the worst situation in terms of early, high prevalences and severe losses in June–July 1967. Some infections were clinical on 20 October 1966 and prevalences of 64 to 80% were reached on 14 December 1966. Although advanced infections (heavy and moderate) prevailed throughout winter and early spring, only 16% of the tray oysters died before 1 June 1967. Because 86% (43 of 50 gapers) of the deaths can be attributed to MSX, one can extrapolate to a very high incidence for the year by adding 16% deaths in winter and spring to 80% infection in May 1966. The years 1964 and 1965 exhibited similar high prevalence levels by mid-winter and severe death rates in the following mortality periods of June–July.

The time pattern of infections returned in 1967 to the original one of late appearance of clinical cases, and infections failed to appear in the spring of 1968. In recent years, note that advanced infections were scarce even in May, and low death rates prevented collection of gapers. A failure to import disease-free, susceptible oysters in the fall of 1969 and 1970, leaves these years to be rated from May–June prevalence data in oysters imported the previous spring. However, a tray of Wreck Shoal oysters (Y65), that had a few infected oysters when imported in September 1970, indicated that late summer infections failed that year.

The year 1971 appeared to be a very light one for MSX infections (Y72). In 1972, MSX failed at Gloucester Point because of excessive freshwater runoff from Tropical Storm Agnes, which reduced salinities drastically. A near failure of infection occurred in late summer 1973 although use of Wreck Shoal oysters with a few infections compromised the data (Y79). Spring-imported oysters in 1973 also failed to show a significant late summer infection in May 1974.

Recovery to normal salinities after the wet years of the early 1970's permitted MSX to develop high levels of infection in 1974 and 1976. However, 1975 was a moderate year for MSX. The unwitting use of infected lots of oysters from Wreck Shoal and Rainbow Rock in the mid-1970's makes the data hard to interpret. Scarcity of seed oysters in the upper James River was the immediate cause of this mistake in monitoring procedure. The period 1977–1979 consisted of mild infection years with prevalences in the 20 to 30% range.

#### *Summary by Years of *H. nelsoni* Activity from Late Summer Infection*

A rating by years of MSX activity in lower Chesapeake Bay from late summer and fall infections is presented in Table 3. The 19 years of records on late summer and fall infection show 8 years with early appearance of infections (fall or winter), 6 years with late occurrence of clinical cases (April or May), and 5 years in which no infection occurred. This contrasts with early summer infection when MSX never failed to be active in lower Chesapeake Bay, and failed only once (1972) at Gloucester Point where presently reported records were obtained. Typically low and high prevalences resulted in corresponding low and high mortalities (Table 3). Prevalences were usually higher than mortalities which may be explained by late regression of infections and by early or late deaths outside the usual June–July mortality period.

The rating of MSX activity by years is based on a subjective combination of prevalences in May, when peak levels of infections usually occurred, and mortalities during June and July. An average year is defined as exhibiting 50% prevalence in May, and 40% mortality in June–July. Years of no infections were excluded in deriving these averages. The reason for no infections in some years is not known. Generally, the years of above-average MSX activity were dry ones, but there is not a strong relationship between salinity and MSX intensity in lower Chesapeake Bay where the disease is endemic and well established.

#### *Comparison of Chronological Events in Early Summer and Late Summer Infection of *H. nelsoni**

To summarize and contrast the timing of events following early versus late summer infection, a chronological list for each is given in Table 4. The additive effects of overlapping infections and mortality periods cannot be determined completely from experiments in open waters with natural infections. This list of events is based upon importation of disease-free oysters at the proper times to limit infections to one period or the other. After one high-mortality period, the surviving population is more resistant to the pathogen. Some persistent infections result in mixing the effects from the two exposure periods.

Fortunately, the late-summer disease sequence is quite discrete in timing of events in some years, permitting good

TABLE 3.

Epidemiological rating of late-summer infections and mortalities of *Haplosporidium nelsoni* (MSX) by years and by timing. Infections occurred in the year of importation and mortalities the following year in June-July.

Year of Importation	Rating of MSX Activity*	Prevalence Level in May (%)	Mortality, Jun-Jul (%)	Timing of first Appearance of Infections**
1961	below average (low mortality)	50	26	late
1962	below average (low prevalence)	30	16	late
1963	average (low mortality)	40-50	29	early
1964	above average	50-60	41	early
1965	above average	60-70	40	early
1966	very heavy	60-80	62 <sup>a</sup> 52 <sup>a</sup>	very early
1967	below average	< 30	30-40	late
1968	none	---	---	---
1969	trace	---	---	---
1970	none	---	---	---
1971	very light	< 20	< 10	very late
1972	none (Agnes flood)	---	---	---
1973	trace	---	< 10	---
1974	above average	60	44	early
1975	below average	30-40	22	late
1976 <sup>b</sup>	above average	60	50	early
1977 <sup>b</sup>	below average	20-30	15	late
1978 <sup>b</sup>	below average	20-30	29	rather early
1979	below average	20-30	40	rather early
1980	?	?		?

\*Based on prevalences (average = 50%) and mortalities (average = 40%).

\*\*"Late" is April or May of following year. "Early" is late fall or winter of same year.

<sup>a</sup>+16% mortality before 1 June 1967.

<sup>b</sup>Early-summer infections nearly failed in 1977 and 1978 after June-July kills.

estimates of prevalences and mortalities. The extent to which the two disease sequences support and depend upon each other as sources of infective particles is uncertain. The mortality period from early summer infection is the infection period for late summer infection. The June-July mortality period from late summer infection is the infection period for early summer exposure. These relationships would have significance only if oysters are the sources of infective stages rather than an alternate host.

#### DISCUSSION

Data on late summer and fall infections and subsequent mortalities caused by the oyster pathogen *Haplosporidium nelsoni* are presented separately from data for early summer infection because of their possible importance in the normal life cycle of the parasite. Disease-free oysters imported

after 1 August and before 1 November acquire infections promptly, but these often do not become clinical by present diagnostic methods (stained cross sections) until April or May of the following year. Then a characteristic June-July mortality occurs during which most infected oysters die, with regression of infections occurring in a few cases. Sparse data suggest that May-June may be the normal time for MSX to sporulate, although failure of sporulation to kill promptly results in the rare cases of spores being found in live oysters at almost any time of the year (Andrews 1979a).

These late-summer-infection patterns of (1) long incubation period, (2) sudden appearance of infections in spring, and (3) distinctive two-month early-summer mortality have similarities in epizootiology to the closely related *Haplosporidium costalis* which causes Seaside

TABLE 4.  
Chronology of events in life cycle of MSX in Virginia.

A. Early-Summer Infections (mid-May to 1 August)	B. Late-Summer Infections (1 August to 1 November)
1. 1 Nov to 1 Jun: Disease-free seed oysters transplanted from James River seed area to private rented grounds in high-salinity waters.	1. 1 Aug to 1 Nov: Importation of disease-free oysters from James River; public oystering season begins 1 October.
2. Mid-May to 1 Aug: Early-summer infection period for MSX. (No failures in 23 years.)	2. Aug–Oct: Immediate infections of imported oysters, but infections remain subclinical or localized for months. (Infections fail some years.)
3. 1 to 15 Jul: Earliest infections become clinical.	3. Late fall and early winter: Clinical infections appear in some years of intensive infection pressure.
4. 1 Aug: Typical time for first MSX-caused deaths.	4. Jan–May: Very few deaths occur even with advanced infections in severe MSX years. Infections gradually become patent and increase in intensity.
5. 1 Sep: Typical peak of mortality from MSX.	5. May: Peak prevalences occur for late-summer infections.
6. 1 Nov: Low temperatures stop deaths of remaining infected oysters.	6. Jun–Jul: Most deaths occur and sporulation occurs rarely.
7. Nov through Jan: Very low death rates ( $< 5\%$ /month).	7. July: Old late-summer infections become mixed with new early-summer infections; often distinguished by intensities.
8. Feb–Mar: Late-winter kill of oysters with advanced infections of MSX. (Some deaths from other causes.)	8. Aug–Sep: The early-summer cohort of infections has become dominant causing late-summer mortalities.
9. Apr–May: Low-mortality period ( $< 5\%$ /month).	
10. Jun–Jul: Last of oysters infected previous June die (some regression of infections may occur in spring).	

Disease of oysters. *Haplosporidium costalis* has a short infection period from about 15 May to 15 July, a long incubation of up to 9 months before clinical infection can be observed, and a short mortality period of about 6 weeks (usually mid-May to 1 July). It sporulates regularly and completely (all plasmodia become sporonts) and kills oysters promptly (Andrews and Castagna 1978, Andrews 1979a).

Some investigators of oyster diseases conclude that MSX must have an alternate or other host (Farley 1967, Ford and Haskin 1982). This concept of the life cycle of MSX is based on failures to achieve artificial infection in laboratories with both MSX and other haplosporidan pathogens of other hosts; e.g., with chitons (Pixell-Goodrich 1915). Only Barrow (1965), working with freshwater snails, has claimed direct transmission of these pathogens. The assumption that spores are the normal method of transmission coupled with the extreme scarcity of MSX spores in oysters in Chesapeake and Delaware bays has also encouraged the view that another host is involved. No alternate host has been found despite intensive examinations of some potential hosts such as mobile blue crabs and fishes which are widespread and would appear to be likely transmitters of the disease (Sprague 1970, 1971, 1978). Almost identical spores (shape and size) have been found in shipworms (Hillman 1979) and Asiatic oysters (Kern 1976). The arguments for and against alternate or other hosts have been discussed (Andrews 1979b), but positive evidence has not been found.

*Haplosporidium costalis* exhibits regular patterns of activity through the year and completes its life cycle by sporulation every year in May–June (Andrews and Castagna 1978). It does not appear to impede oyster activity for about 8 months of the year, yet it develops intensive infections and kills oysters rather rapidly in the spring. It seems to be an adapted parasite that limits its damage to the

host population. It could be a useful model for what may be expected in the life cycle of MSX, if that pathogen were parasitizing a host population that had developed enough resistance to moderate the pathogenicity. In contrast, MSX infects oysters over a long period exceeding 5 months, and it kills up to 75% of a population of previously unselected oysters in 2 years of exposure. It would be helpful in understanding the pathogenicity of MSX to find the geographical area (probably in Asia; re: Kern 1976) where it evolved and also the endemic host species (presumably *Crassostrea gigas*) in that area. Epidemics of human diseases and introduction of new diseases of oysters in France (Andrews 1981) have shown that an exotic pathogen thrust suddenly on a previously unexposed race of the host species can become catastrophic in its effects.

Several-hundred-thousand oysters have been sectioned over a 25-year period to monitor MSX along the mid-Atlantic coast. Therefore, it appears unlikely that the missing links of the life cycle will be found by looking further at routinely stained sections. The infective stages are not known and their possible sources and abundance are disputed. The factors that trigger pathogenicity and sporulation are also undefined, and even the timing of infections is unexplained. The change in infection and mortality patterns after 1 August each year may reflect reduced abundance of infective particles, for there is no significant change in environmental factors such as salinity and temperature at this mid-summer time. If spores releasing sporoplasms are required for infections, there must be another host providing them, for oysters along the Atlantic coast do not produce enough spores to sustain the infections observed. However, in an aquatic environment it is quite possible that plasmodia can survive and infect oysters when strained by gill tissues from currents produced while the oyster is feeding.

Complete elucidation of the life cycle of MSX, and explanation of the variable incubation periods before clinical infections are observed, require successful artificial infection under controlled laboratory conditions. This is the chief stumbling block in understanding MSX epizootiology and predicting consequences of environmental changes such as the record high salinities of 1980–1981 in Chesapeake Bay. Resistant oysters are available to compare the effects of various abundances of infective particles if induced infections were possible (Haskin and Ford 1981).

*Haplosporidium costalis* (SSO) offers the best choice of a haplosporidan pathogen to achieve artificial infection in oysters. All stages of the life cycle are known, presumably, and are readily available at known times. It infects oysters during the mortality season when spores are available. It is confined to high-salinity ( $> 25$  ppt) waters and can be readily controlled by movement of oysters to low-salinity waters. The long incubation period constitutes a problem for artificial infection. In natural waters, reduction of SSO cases and interference by MSX were often severe in recent

years. Hunting for an alternate host is a difficult approach to the life cycle of MSX or SSO because the infective particles are water borne and infections do not require close proximity of the donor species. The wide distribution of MSX and complete coverage of all localities within the endemic area suggests high abundance and wide distribution of the host species that provides infective particles. Oysters certainly fit these requirements.

Drastic changes in the populations of oysters in lower Chesapeake Bay, after the sudden catastrophic appearance of MSX, did not change the levels of MSX activity in subsequent years. Over large areas of lower Chesapeake Bay, susceptible oysters exhibited close similarities in timing of epizootiological events, including infection levels and mortalities for both short-term and annual periods. Some variations in intensities of MSX activity occurred from year to year, but the astonishing facts were its persistence and its regularity regardless of oyster abundance. It is possible that a closer look at the epizootiology of late-summer MSX infection may provide clues to the life cycles of other haplosporidans.

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## THE EFFECTS OF PARASITISM BY *PERKINSUS MARINUS* ON THE FREE AMINO ACID COMPOSITION OF *CRASSOSTREA VIRGINICA* MANTLE TISSUE

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**ABSTRACT** Seventeen free amino acids were detected from oyster mantle tissue. Taurine, serine\* (serine plus asparagine and glutamine), glutamic acid, glycine, alanine, and  $\beta$ -alanine were found in concentrations greater than 30  $\mu\text{mol/g}$  dry wt. Moderate concentrations (10 to 30  $\mu\text{mol/g}$  dry wt) of aspartic acid and arginine were detected. Threonine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, and histidine occurred in concentrations of less than 10  $\mu\text{mol/g}$  dry wt.

Taurine and aspartic acid concentrations increased with increasing parasitism by *Perkinsus marinus*; the concentrations of glycine, alanine, isoleucine, leucine,  $\beta$ -alanine, arginine, and total free amino acids decreased with increasing parasitism.

Ratios of specific free amino acids with taurine showed a closer correlation with the level of parasitism than similar correlations of parasitism with individual amino acids. A correlation between the level of parasitism by *P. marinus* and the molar ratio of taurine-to-glycine showed a particularly close relationship ( $r = 0.90$ ,  $P < 0.001$ ).

This study corroborates the usefulness of the taurine-to-glycine ratio as a biochemical measure of stress. The close relationship between this ratio and level of parasitism suggests that *P. marinus* may be the agent of stress.

### INTRODUCTION

Since its original description as *Dermocystidium marinum* by Mackin et al. (1950) and the development of a relatively easy and effective method for its detection by Ray (1952, 1966), many studies have been conducted on the parasite now called *Perkinsus marinus* (Levine 1978). Numerous studies (Mackin et al. 1950; Mackin 1951, 1955, 1961; Hewatt and Andrews 1955; Andrews and Hewatt 1957; Quick and Mackin 1971) have shown that the parasite is more prevalent at high salinities and high temperature. Other works have implicated *P. marinus* as a cause of mortalities in its oyster host, *Crassostrea virginica* (Gmelin) (Ray and Mackin 1954, Andrews 1965). On the basis of histological studies, Mackin (1951) found that early stages of infection were characterized by inflammation followed by fibrosis and extensive lysis of host tissues as the infection progressed. Ray et al. (1953) showed that the mean weight of heavily infected oysters was 33% less than that of uninfected controls and suggested that lysis of tissues was one of the major processes resulting in the loss of weight of oysters.

In contrast to our knowledge of the ecological preferences and histological consequences of infection by *P. marinus*, little is known of the biochemical effects of this parasite on its host. Stein and Mackin (1955) found significantly more "pigment cells" in heavily infected oysters than in uninfected or lightly infected ones. Granules within the pigment cells were histochemically shown to be lipofuscin. The increased amount of lipofuscin suggests that a change in fat metabolism is associated with parasitism.

Although changes in the free amino acid composition of host organisms are known to be associated with parasitism (Feng et al. 1970, Boctor 1979, Vivares et al. 1980), no information exists on the effects of infection by *P. marinus* on the free amino acid composition of oysters.

### MATERIALS AND METHODS

Oysters of the same approximate size were collected by hand in about 1 m of water from Offats Bayou (Galveston, TX) during October 1980. Water salinity (determined with an American Optical refractometer) and temperature were recorded. The length of the right valve (umbo-to-bill distance) was measured. The oysters were sexed and the gonads were inspected for infection by trematodes (*Bucephalus* sp.). Sexing and inspection for *Bucephalus* sp. involved cutting the oyster across the gonad and blotting gonadal material onto a slide for microscopic examination. One piece of mantle tissue was used to determine the intensity of infection by *P. marinus* (Ray 1966). The intensity of infection was scored as an integer from 0 (uninfected) to 6 (heavily infected), according to the criteria of Quick and Mackin (1971). Another piece of mantle tissue was frozen at  $-20^{\circ}\text{C}$ . After the intensity of infection was determined, 10 tissue samples were chosen for amino acid analysis. Samples were chosen such that the entire infective range was represented.

Free amino acids were extracted by the method of Kittredge (1974). Individual amino acids were identified and quantified with the use of an amino acid analyzer (American Instrument Company). The amino acids were identified by the order of elution from an ion-exchange column. Oyster samples were compared to a Pierce protein hydrolysate of known amino acid composition. As the individual amino acids elute from the column, they react

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with orthophthaldialdehyde (OPA), creating a fluorescent product (Lee and Drescher 1978). The absorbance of the fluorescent product is then measured. The amino acids were quantified by measuring the area under each absorbance peak. A correction factor was applied to the data to compensate for the variable extent to which different amino acids react with OPA. The corrected amino acid peak areas were then converted to concentrations. The concentrations of amino acids were calculated by reference to an internal standard, norleucine, which was added to each sample in a known quantity.

Seventeen amino acids were present in sufficient quantity to be reliably reported. Proline could not be detected. Tyrosine, tryptophan, and cysteine were not found or were present in negligible quantities. Asparagine and glutamine coeluted with serine in this system. Serine is, therefore, reported as serine<sup>+</sup>.

### RESULTS

Two of the 10 oysters selected for amino acid analysis were males; the remainder were females. No evidence of infection by *Bucephalus* sp. was found in the gonads of

any of the oysters collected. The oysters ranged in length from 9.2 to 12.1 cm. The water salinity and temperature at the time of collection were 26.0 ppt and 28.2°C, respectively.

Seventeen free amino acids were detected from oyster mantle tissue (Table 1). High concentrations ( $\bar{X} > 30 \mu\text{mol/g}$  dry wt) of taurine, serine<sup>+</sup>, glutamic acid, glycine, alanine, and  $\beta$ -alanine were found. Moderate concentrations ( $\bar{X} = 10$  to  $30 \mu\text{mol/g}$  dry wt) of aspartic acid and arginine were present. Threonine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, and histidine occurred at concentrations of less than  $10 \mu\text{mol/g}$  dry wt.

Taurine and aspartic acid increased with increasing parasitism by *P. marinus*; glycine, alanine, isoleucine, leucine,  $\beta$ -alanine, arginine, and total free amino acids decreased with increasing parasitism (Table 1).

Ratios of specific free amino acids to taurine showed a closer correlation with the level of parasitism than similar correlations of parasitism with individual amino acids. A correlation between the level of parasitism and the molar ratio of taurine to glycine showed that a close relationship exists ( $r = 0.90$ ,  $P < 0.001$ , Figure 1).

TABLE 1.  
Free amino acid concentrations ( $\mu\text{mol/g}$  dry wt) in the mantle tissue  
of *Crassostrea virginica* at various levels of  
parasitism by *Perkinsus marinus*.

Amino Acid	Disease Code Number										$\bar{X} \pm \text{SD}$	Level of Significance <sup>3</sup>
	0	0	0	1	2	3	4	5	6	6		
Taurine	74.4	68.9	107.5	111.2	60.7	101.9	61.6	139.8	139.5	135.1	100.1 $\pm$ 32.0	* <sup>4</sup>
Aspartic acid	13.6	11.6	14.8	14.1	9.6	12.0	7.0	23.7	26.9	26.3	16.0 $\pm$ 7.1	** <sup>4</sup>
Threonine	2.3	1.0	2.7	2.3	1.2	2.0	0.8	0.8	2.6	1.3	1.7 $\pm$ 0.8	NS <sup>5</sup>
Serine <sup>+</sup> 1	32.8	54.1	35.8	29.2	38.4	63.5	48.7	22.1	13.5	17.9	35.6 $\pm$ 16.1	NS
Glutamic acid	25.4	56.3	40.9	51.5	32.0	53.2	32.7	50.2	23.7	26.4	39.2 $\pm$ 12.7	NS
Glycine	62.8	55.1	67.1	48.9	36.0	30.3	18.4	36.1	16.7	19.1	39.1 $\pm$ 18.6	*** <sup>4</sup>
Alanine	48.0	92.1	112.2	158.0	86.6	56.3	50.4	52.0	30.1	30.0	71.6 $\pm$ 40.6	**
Valine	3.9	3.2	0 <sup>2</sup>	0	0	0	0	0	0	1.0	0.8 $\pm$ 1.5	NS
Methionine	1.8	2.2	0	2.4	1.2	0.8	1.2	0.8	0	1.6	1.2 $\pm$ 0.8	NS
Isoleucine	3.1	2.4	2.7	2.0	3.9	3.8	0	1.5	0	1.0	2.0 $\pm$ 1.4	**
Leucine	6.8	6.8	7.5	6.1	7.2	6.1	1.6	2.4	4.6	2.6	5.2 $\pm$ 2.2	**
$\beta$ -alanine	66.0	72.7	87.4	154.0	50.4	53.9	62.6	41.9	28.5	36.9	65.4 $\pm$ 35.7	*
Tyrosine	4.4	2.8	3.2	0	4.0	7.7	0	3.5	0	0	2.6 $\pm$ 2.6	NS
Phenylalanine	2.7	2.0	3.4	0	3.6	3.8	0	3.5	0	0	1.9 $\pm$ 1.7	NS
Lysine	6.8	12.2	10.6	0	8.5	10.1	2.7	4.3	2.7	2.9	6.1 $\pm$ 4.1	NS
Histidine	5.9	12.1	0	7.5	1.0	5.2	2.8	2.8	2.8	1.5	4.1 $\pm$ 3.6	NS
Arginine	10.1	18.2	22.7	18.2	14.1	15.1	10.8	12.7	7.3	8.3	13.7 $\pm$ 4.9	**
Total amino acids	370.8	473.7	518.5	605.4	358.4	425.7	301.3	397.9	298.9	311.9	406.3 $\pm$ 101.2	**

<sup>1</sup> Serine<sup>+</sup> refers to serine plus asparagine and glutamine.

<sup>2</sup> Zeros (0) represent trace levels.

<sup>3</sup> Determination of level of significance is based upon a correlation between the level of parasitism and the free amino acid concentrations.

<sup>4</sup> \*, \*\*, \*\*\* refer to significance between level of parasitism and amino acid concentration at  $P < 0.10$ ,  $P < 0.05$ , and  $P < 0.01$  levels, respectively.

<sup>5</sup> NS = not significant.

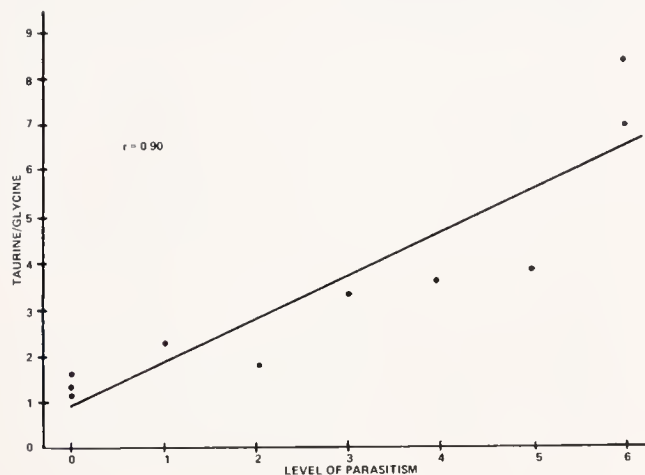


Figure 1. The relationship between level of parasitism by *P. marinus* and the molar ratio of taurine-to-glycine in *C. virginica* mantle tissue.

### DISCUSSION

It appears that the principle effect of the parasite is to decrease specific as well as total free amino acids (Table 1). Because nothing is known about the free amino acid composition of *P. marinus*, it is impossible to ascertain its contribution to the free amino acid pool of infected tissue. Such a contribution, however, is likely to be small. The increase in aspartic acid, and especially taurine (Table 1), may represent an effort on the part of the oyster to maintain osmotic balance by replenishing free amino acids depleted by the parasite (Feng et al. 1970).

The importance of free amino acids in maintaining osmotic balance is well documented. Lange (1963), for example, found that the concentrations of free amino acids in *Mytilus edulis* Linnaeus increased with increasing salinity. Taurine, relative to total ninhydrin positive substances, increased most dramatically with increasing salinity, suggesting that taurine has a sparing effect on the use of essential amino acids in osmoregulation. Lynch and Wood (1966) showed that the total free amino acid concentration in adductor muscle tissue of *C. virginica* was higher at elevated salinities.

In the present study, only some of the variability in changes of free amino acids is attributable to the effects

of parasitism. A linear regression between the level of parasitism and the total free amino acid concentrations explained only about 46% ( $r^2 = 0.46$ ) of the variability in the data. Sources of variability in free amino acid pools (besides parasitism) include: the nutritional state of the organism (Schafer 1961, Bayne et al. 1976, Sansone et al. 1978), natural seasonal changes (Jeffries 1972, Zurburg and DeZwaan 1981), responses to changes in environmental salinity (Lange 1963, Lynch and Wood 1966, Baginski and Pierce 1977), and effects of pollutants (Schafer 1961, 1963; Jeffries 1972; Roesijadi and Anderson 1979).

A close correlation was found between the level of parasitism and the molar ratio of taurine-to-glycine (Figure 1). Jeffries (1972) proposed the use of the taurine-to-glycine ratio as an indicator of stress in *Mercenaria mercenaria* (Linnaeus). When the molar ratio of taurine-to-glycine is less than 3, the population is considered normal; if it is greater than 3, stress is indicated. The data of Feng et al. (1970) show ranges in the ratio from 0.5 to 2.0 ( $\bar{X} = 1.2$ ) for normal *C. virginica*, and 1.3 to 9.1 ( $\bar{X} = 4.0$ ) for infected oysters. Lee et al. (1980) reviewed the literature on the taurine-to-glycine ratio and suggested the use of the ratio as an index of stress in certain species of bivalves. Jeffries (1972) claimed that the change in the ratio represented a general reaction of the clam to disease trauma, laboratory conditions, pollutants, starvation, and seasonal variations in the environment. Furthermore, Jeffries believed that the magnitude of the ratio suggested causative factors. Values between 3 and 5 resulted from physical-chemical abnormalities in the environment; values  $> 5$  indicated disease. In this study, taurine-to-glycine ratios of 3.4 to 3.9 were found in oysters that were moderately infected. Ratios of 7.1 and 8.4 were found in heavily infected oysters (Figure 1).

The close correlation between parasitism by *P. marinus* and taurine-to-glycine ratios in mantle tissue of *C. virginica* corroborates the usefulness of this ratio as a biochemical measure of stress and suggests the possibility that *P. marinus* was the agent of stress in this study.

### ACKNOWLEDGMENTS

The authors express their appreciation to Sammy M. Ray, James Kittredge, and James Dodson for their assistance. This study was made possible by support from the Texas A&M Sea Grant marine fellowship program, Texas A&M University at Galveston, and the Gulf and South Atlantic Fisheries Development Foundation, Inc.

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## THE ACCEPTABILITY AND DIGESTIBILITY OF MICROCAPSULES BY LARVAE OF *CRASSOSTREA VIRGINICA*

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**ABSTRACT** The acceptability and digestibility of microcapsules with gelatin-acacia and nylon-protein walls to larvae of *Crassostrea virginica* were assessed. Larvae were observed to ingest and digest the microcapsules. Gelatin-acacia microcapsules were more digestible than the nylon-protein microcapsules. Results indicated that both types of microcapsules supported some growth of larvae. Larvae fed cod liver oil encapsulated by gelatin-acacia walls grew as rapidly as larvae fed algae. Results also indicated that microcapsule concentration affected growth rate.

### INTRODUCTION

A major difficulty in the development of commercial culture systems for molluscan and crustacean larvae is the dependence upon supplies of live organisms for food. This dependence has also obstructed investigations into the nutritional requirements of many bivalve molluscs and crustaceans during their planktonic larval life, although some valuable information about larval nutrition has been gained in the last decade.

Artificial food particles are acceptable to a wide range of filter feeders (Ling 1969, Paffenhofer and Strickland 1970, Jones et al. 1972). However, those particles could be susceptible to disintegration and associated bacterial contamination. One solution to these problems is to use an encapsulated diet. Moreover, if the diet can be defined biochemically, the technique of microencapsulation can be used to investigate the exact nutritional requirements of the animals under culture conditions.

The type of microcapsule that can be used successfully in feeding experiments will be dependent on the mode of feeding of the animals. Bivalve larvae and adults are filter feeders and ingest their food intact. Selection of food particles depends on size, surface properties, and weight of the particle (Ukeles 1971, Owen 1974). Therefore, the test of the acceptability of different types of microcapsules to the animal is important to justify future experiments with encapsulated diet components to evaluate growth and survival. It is also important to demonstrate that microcapsules which are acceptable in terms of ingestion and retention to the bivalves can be digested.

Gelatin-acacia microcapsules are suitable for the presentation of dietary lipids to larvae of *Crassostrea gigas* (Langdon 1980). Previous investigators suggested that lipids play a significant role in the metamorphosis and development of oyster larvae (Helm et al. 1973, Holland and Spencer 1973, Holland 1978, Chu and Dupuy 1980). Consequently, gelatin-acacia microcapsules filled with cod liver oil were used in these feeding experiments. Cod liver oil is rich in

highly unsaturated fatty acids (Ackman and Burgher 1964) and has a fatty acid composition quite similar to that found in the protocol algal diet (a combination of *Chlorella* sp., *Pyramimonas virginica*, and *Pseudoisochrysis paradoxa*) used in this laboratory as a standard food source for larvae of *Crassostrea virginica* (Chu and Dupuy 1980).

Jones and his colleagues (Jones et al. 1974, Gabbott et al. 1975, Jones and Gabbott 1976, Jones et al. 1979a, Jones et al. 1979b) successfully encapsulated artificial food particles in nylon-protein microcapsules to study the nutritional requirements of crustacean larvae. It was apparent, therefore, that nylon-protein walled microcapsules could be used to provide protein, lipid, and carbohydrates to oyster larvae. In this paper results of the assessment of the acceptability to and digestibility by larvae of *C. virginica* are reported for microcapsules with gelatin-acacia and nylon-protein walls.

### METHODS AND MATERIALS

#### *Microcapsules and Diet*

Gelatin-acacia microcapsules were prepared using the methods of Green and Schleicher (1957). Cod liver oil containing vitamins A and D (E. R. Squibb and Sons Inc., Princeton, NJ) was encapsulated for feeding experiments. The mean diameter of the microcapsules was  $6.0 \pm 1.8 \mu\text{m}$  ( $\bar{x} \pm \text{SD}$ ,  $n = 25$ ). Stained gelatin-acacia microcapsules were prepared by dissolving Sudan Red in lipid before encapsulation (approximately 1 to 2 mg/ml lipid). Vitamins B<sub>1</sub>, B<sub>2</sub>, and B<sub>12</sub> were supplied in the diet by mixing B<sub>1</sub> and B<sub>2</sub> with the lipid and dissolving the B<sub>12</sub> in the solution of gelatin-acacia prior to microencapsulation. All gelatin-acacia microcapsules, except those fed to the larvae immediately after manufacture, were autoclaved at 121°C (1.053 kg/cm<sup>2</sup> [15 psi] pressure) for 15 minutes and stored in the refrigerator. Autoclaving may have somewhat reduced the vitamin content due to heat lability; vitamin A is considered heat labile at 121°C while vitamins B<sub>1</sub>, B<sub>2</sub>, B<sub>12</sub>, and D are not.

Nylon-protein microcapsules were prepared with a modification (Jones and Gabbott 1976) of the polymerization procedure described by Chang et al. (1966). The mean diameter of these microcapsules was  $6.1 \pm 1.95 \mu\text{m}$  ( $n = 25$ ). Whole chicken egg homogenate was mixed with an equal volume of 15% dextrose and 5% cholesterol in distilled water and was incorporated into the nylon-protein microcapsules. Nylon-protein microcapsules were stained by adding Blue dextran to the egg-water mixture (8 mg of Blue dextran for 15 ml of mixture). A summary of the general characteristics of these two types of microcapsules is shown in Table 1.

TABLE 1.  
Some properties of gelatin-acacia and  
nylon-protein microcapsules.

Microcapsules	Gelatin-acacia	Nylon-protein
Size range (diameter)	3 to 8.25 $\mu\text{m}$	3 to 9 $\mu\text{m}$
Mean	6.0 $\mu\text{m}$	6.1 $\mu\text{m}$
Standard deviation	1.8	1.8
n	25	25
Filling	Cod liver oil and fat-soluble components (e.g., vitamins)	Egg protein or haemoglobin, cholesterol and dextrose
Permeability	Permeable	Semi-permeable
Potential use	To supply lipid, fat-soluble micronutrients	To supply protein, lipid, and carbohydrates

#### Larval and Algal Culture

Methods used to induce spawning and for embryo culture were those of Dupuy et al. (1977). After spawning, all eggs were pooled and counted before fertilization. About  $12.5 \times 10^6$  fertilized eggs were placed in each 250-l fiberglass larval tank. When the cultures were maintained at temperatures of 27 to 28°C, the larvae reached the straight-hinge stage 18 to 24 hours after fertilization. The methods of Dupuy et al. (1977) were also used in rearing and feeding the oyster larvae.

One algal species, *Pseudoisochrysis paradoxa*, used as part of our protocol diet for bivalve larvae was cultured at 16 to 19°C in 40-l carboys containing filtered and pasteurized estuarine water enriched with N<sub>2</sub>M medium (a mixture of Ketchum & Redfield's solution A and B, sodium molybdate solution, Arnon's micronutrient solution), and a horse manure extract mixture (Dupuy et al. 1977). *Pseudoisochrysis* was grown under continuous illumination from one warm white and one Gro-Lux fluorescent lamp. Continuous aeration provided circulation in the cultures.

#### Feeding Experiment

Two feeding experiments with larvae of *C. virginica* were carried out in the laboratory. Larval density in all

feeding experiments was 5 to 6 larvae/ml. Larvae that were fed *P. paradoxa* (the other two species of the protocol diet were unavailable), and starved larvae served as controls for all feeding experiments. Seawater filtered through 10 and then 1  $\mu\text{m}$  Cuno cotton filters was used throughout these experiments.

**Feeding and Digestion Activity.** Stained gelatin-acacia microcapsules and nylon-protein microcapsules were fed to 2-day-old larvae in 300-ml glass beakers. Microcapsules were fed to larvae each day for 2 days after which the larvae were held for an additional 3 days in clean water. The seawater in the beakers was changed every other day, prior to feeding if the larvae were fed. Beakers containing larvae were covered and held at room temperature (26–27°C). Samples of larvae were observed with a Zeiss standard UPL inverted microscope 24 and 48 hours after the last feeding to observe the contents of the digestive system. Photographs were taken of the same sample after preservation in a 0.5% formalin solution. A Leitz Ortholux microscope with variable phase contrast optics and a Reichert camera were used with Ektachrome film (ASA 160, tungsten).

**Growth Experiment.** The purpose of the growth experiments was to determine a suitable range of microcapsule concentration to use in subsequent experiments. Growth was the definitive indicator of digestion and utilization of microcapsules.

1. Straight-hinge oyster larvae were grown in 250-l larval tanks with three different concentrations of gelatin-acacia microcapsules containing cod liver oil: 500, 1,600, and 5,000 microcapsules/ml. Starved larvae and larvae fed with *P. paradoxa* were used as controls for this experiment. Some gelatin-acacia microcapsules containing cod liver oil were supplemented with vitamins B<sub>1</sub>, B<sub>2</sub>, and B<sub>12</sub>. Arbitrarily, the ratio of microcapsules without vitamins to those with vitamins was 6:1. Microcapsules were added to the tanks every day and the seawater was changed every second day. The number and size of the larvae were determined on days 3, 5, 11, 13, and 17. Larvae were concentrated (50–250/ml) for counting; the anterior to posterior length of 20 of these larvae were measured.

2. Straight-hinge larvae were cultured in 300-ml glass beakers with different concentrations (50, 100, 200, 500, 1,000, and 5,000) of microcapsules/ml. Cod liver-filled microcapsules were added every day and the seawater changed every second day. Twenty larvae were measured at 16 days.

## RESULTS

#### Feeding and Digestion Activity

Larvae were observed to ingest and digest both gelatin-acacia and nylon-protein microcapsules. Sudan Red-stained gelatin-acacia microcapsules in the position of the stomach and digestive diverticular were observed to fade during the first 24 hours after feeding was terminated, and completely

disappeared within 48 hours. Approximately 72 hours elapsed for the larvae to completely digest the nylon-protein microcapsules. Microcapsule-fed larvae appeared healthy and vigorous throughout the test. In this feeding experiment, both types of microcapsules supported some growth. The "starved" larvae stayed in the straight-hinge stage throughout the experiment (Figure 1a) while the microcapsule-fed larvae developed to umbo stage (Figures 1b and 1c).

#### Growth Experiment

Growth rates of oyster larvae cultured in hatchery-size larval tanks on several diets are shown in Figure 2. Microcapsule-fed larvae grew as rapidly as those fed with the alga, *P. paradoxa*, until about day 11, and grew much better than the starved control larvae. Survival was 33% for all treatments with the exception of the 5,000-microcapsule/ml concentration which had less than 10% survival at day 13.

Some growth was evident for every concentration of microcapsules used; growth was reasonably constant above 500 microcapsules/ml (Figure 3) based on results for 16-day-old larvae grown in 300-ml beakers. Least squares analysis of length of 16-day-old larvae and capsule concentration gave a correlation coefficient of 0.72. Larvae grown in the larval tanks showed a similar trend in response to microcapsule concentrations below 2,000-microcapsule/ml (Figure 4). The reduced growth rate at 5,000 microcapsules/ml could not be explained.

#### DISCUSSION

Both the gelatin-acacia and nylon-protein microcapsules were acceptable to larvae of *C. virginica*. Nylon-protein microcapsules were not as digestible as the gelatin-acacia microcapsules presumably because the nylon-protein wall was formed by cross linkage between nylon and protein. The nylon-protein wall is, therefore, less susceptible to attack by digestive enzymes than the gelatin-acacia wall. Jones and Gabbot (1976) have shown that if the nylon content is decreased, the wall becomes more susceptible to proteolytic breakdown. The nylon content can be diminished by reducing the concentration of 1,6-diaminohexane during preparation of the capsules.

It should be emphasized that these experiments were set up primarily to test the acceptability and digestibility of these two types of microcapsules; detailed consideration was not given to requirements for optimal growth. It was interesting, therefore, to find that gelatin-acacia microcapsules filled with cod liver oil were supportive of larval growth and development. Other investigators (Jones et al. 1974, Gabbot et al. 1975, Jones and Gabbot 1976, Jones et al. 1979a, Jones et al. 1979b) also reported that nylon-protein capsules containing protein, starch, and cholesterol supported growth of both the brine shrimp *Artemia*, and the Japanese oyster *Crassostrea gigas*. In our experiments, gelatin-acacia capsules contained only lipid, with the exception of the small amount of protein in the gelatin and

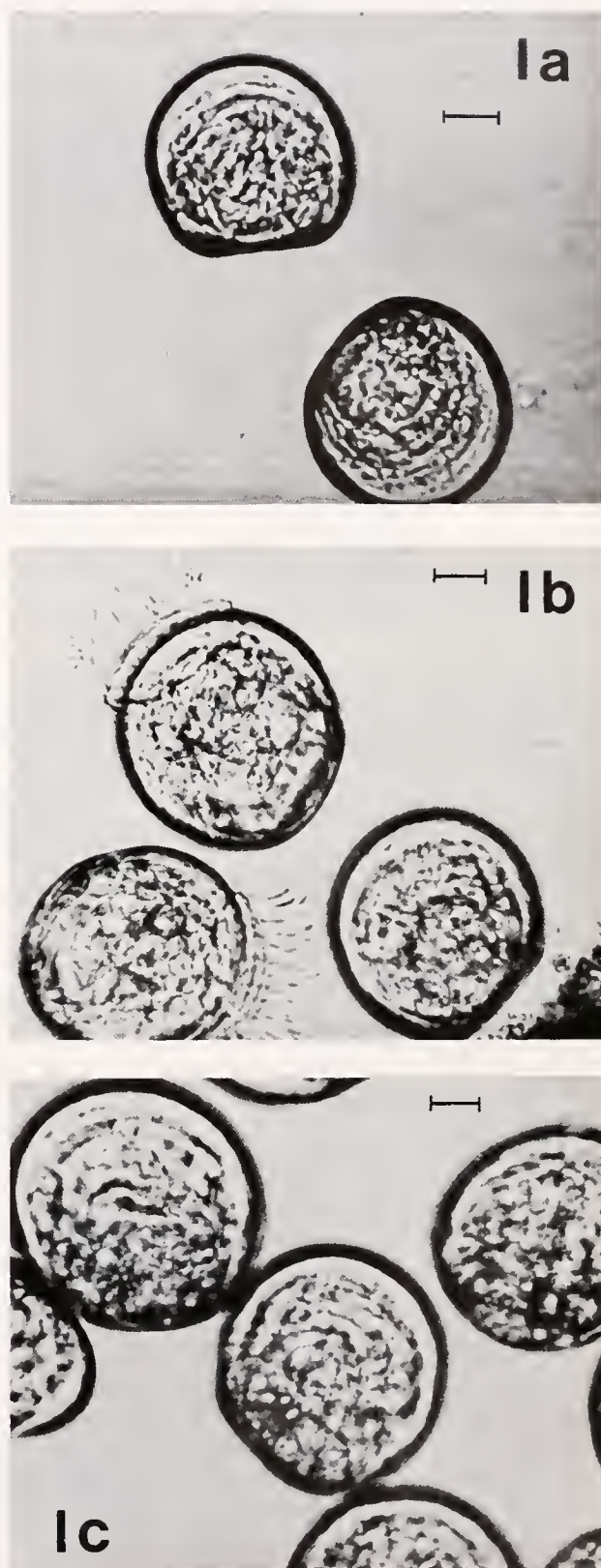


Figure 1. Photomicrographs of 4-day-old oyster larvae: (a) "starved" controls; (b) those fed with gelatin-acacia microcapsules; (c) those fed with nylon-protein microcapsules. (Note that the fed larvae progressed to the umbo stage.) Bar = 20  $\mu$ m.

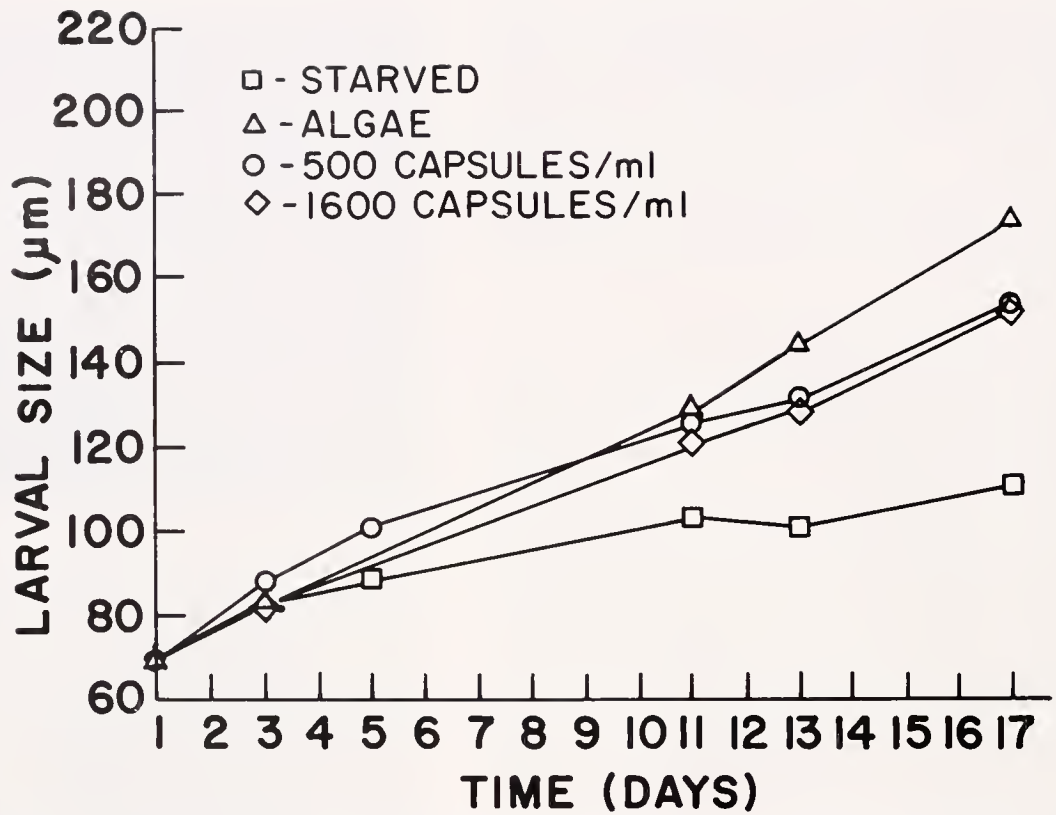


Figure 2. Growth of oyster larvae under different feeding conditions. Larvae were raised in hatchery-size tanks.

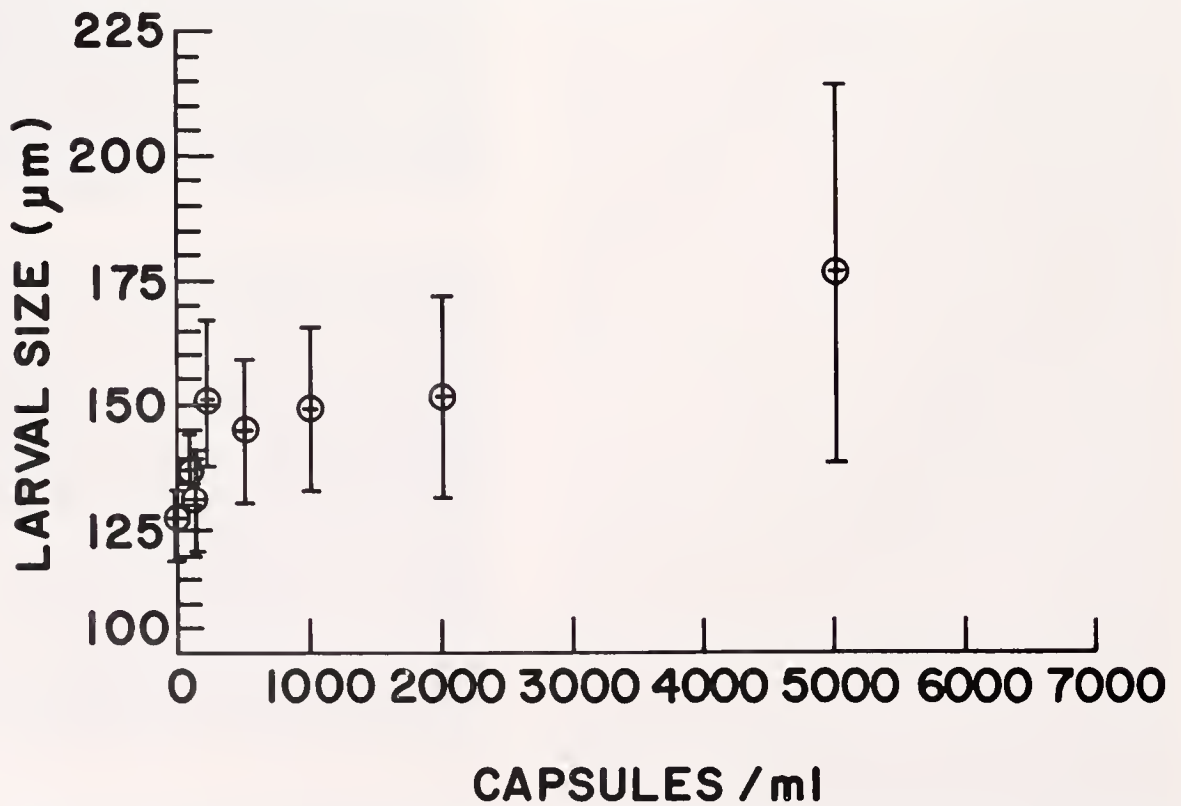


Figure 3. Size of 16-day-old larvae in 300-ml beakers versus concentration of microcapsules. (Standard deviation is indicated by the vertical bars; n = 20.)

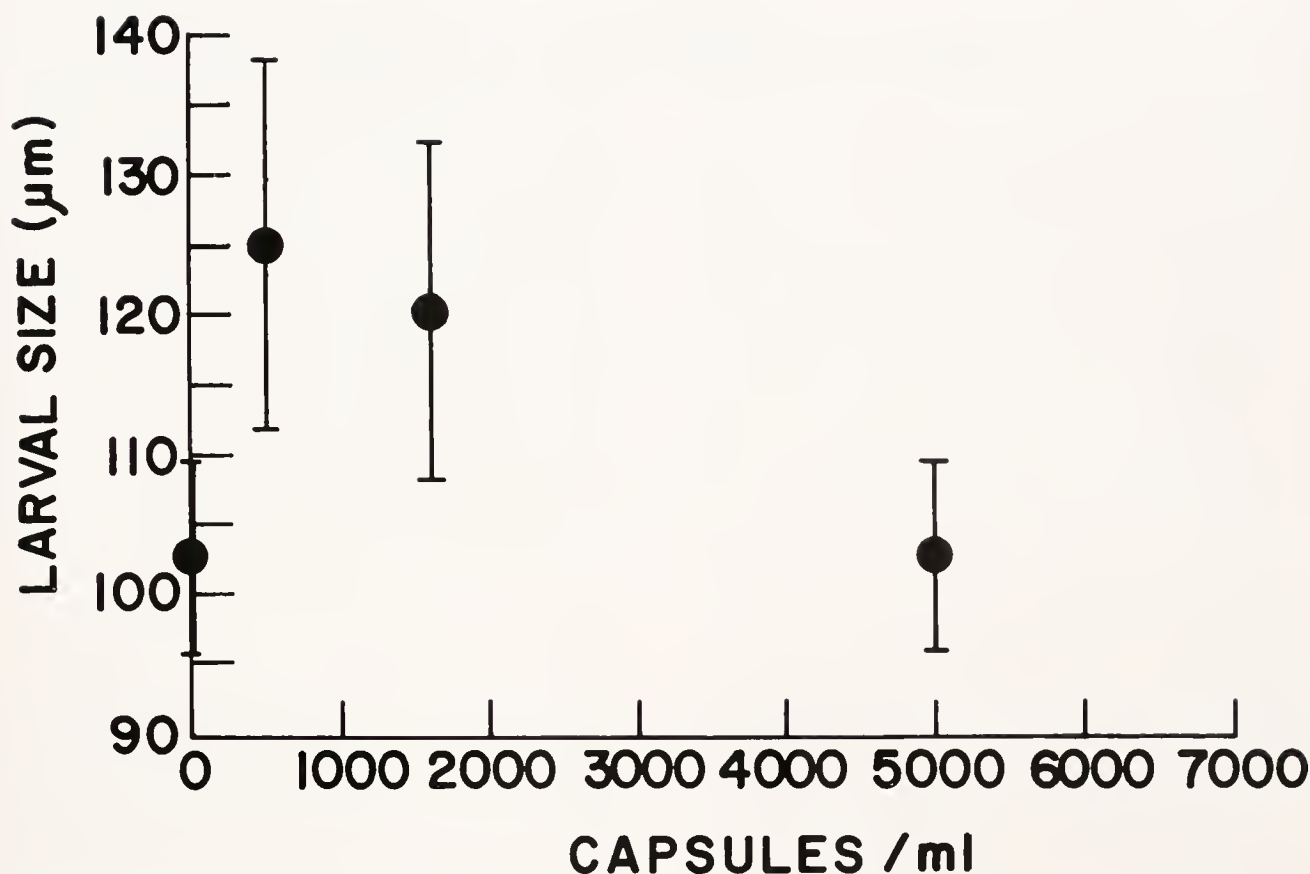


Figure 4. Size of 11-day-old larvae in hatchery-size tanks versus concentration of microcapsules. (Standard deviation is indicated by the vertical bars;  $n = 20$ .)

carbohydrate in the acacia. We anticipate better growth when optimal proportions of lipid, protein, and carbohydrate are encapsulated. There are indications that fatty acids may play a significant role in the metamorphosis and development of oyster larvae (Helm et al. 1973, Holland and Spencer 1973, Holland 1978, Waldoek and Nascimento 1979, Chu and Dupuy 1980). Increasing the supplement of lipid which contains large amounts of long chain polyunsaturated fatty acids (e.g., 22:5w3 and 22:6w3) in the diet could be a promising approach.

Because it is unlikely that vitamins would be present in sea water in sufficient quantity for growth, supplements of  $B_1$ ,  $B_2$ , and  $B_{12}$  were provided. Vitamin  $B_{12}$ , which is water soluble and may leach out during encapsulation, was retained in part by the gelatin-acacia capsules. It is bright red in color and the capsules with  $B_{12}$  were slightly pink.

Gelatin is very susceptible to bacterial attack and bacteria may be attached to the capsule walls. Although bacterial contamination could not have been the source of the bulk nutrients, they may have been the source of trace materials.

It is a disadvantage that the gelatin-acacia wall is permeable and the nylon-protein wall is semi-permeable to small

molecules. Only water-insoluble and macromolecular components of the diet can be contained within such capsule membranes without loss. It would be ideal to produce a capsule with double walls because that type of capsule might be suitable for the encapsulation of both low-molecular weight and water-soluble components (e.g., amino acids and vitamins) as well as lipids. In this approach the aqueous solution would be encapsulated within the lipid before the second outer wall is formed.

#### ACKNOWLEDGMENTS

This work was sponsored by the Office of Sea Grant, NOAA, U.S. Department of Commerce, under Grant No. NA81AA-D-00025, and the Virginia Sea Grant Program through Project No. R/A-10. The U.S. Government is authorized to produce and distribute reprints for governmental purposes notwithstanding any copyright that may appear herein. The authors thank Ms. Beverly Barrett, Ms. Terri Stahl, and Mr. Jeffery Thompson for technical assistance.

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## DESIGN, CONSTRUCTION, AND OPERATION OF A REARING CHAMBER FOR SPAT OF *CRASSOSTREA VIRGINICA* (GMELIN)

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**ABSTRACT** The design, construction, and operation of a molluscan rearing chamber is described in which a continuous flow of filtered, temperature-controlled, ultraviolet-treated seawater washes the mollusks that are suspended on a screen of appropriate size. Feeding may take place at programmed intervals, and the critical testing of nutritional or inimical materials may be conducted under controlled conditions. Construction of the chambers is not difficult and the design may be modified to accommodate various population sizes.

To demonstrate the long-term operation of the chambers, spat of *Crassostrea virginica* were reared on different algal diets. The chambers functioned effectively, oysters increased in weight, and there were no mortalities in oysters fed useful diets during 13 weeks of observation. Growth of oysters was similar and rapid when fed *Tetraselmis maculata* and *Thalassiosira pseudonana*, less rapid when fed *Dunaliella euchlora*, and decreased still further with *Phaeodactylum tricornutum* as a food source. Unfed oysters and those fed *Chlorella autotrophica* showed only small increases in weight during the first 4 weeks of the experiment, but thereafter there was no change.

### INTRODUCTION

Critical determinations of the effects of various nutrient sources, as well as inimical agents, upon growth and viability of bivalve mollusks could be enhanced if containers for animals were designed to contend with problems inherent to the culture of bivalves. Previous studies were conducted in a wide variety of containers, including basins in which seawater was periodically emptied and refilled (Carriker 1961, Gillespie et al. 1964, Walne 1970, Anderson and Anderson 1975), trays suspended in estuarine waters (Castell and Trider 1974, Eldridge et al. 1976) or maintained in the laboratory where seawater flows over the trays (Haven 1965), and closed-cycle recirculating seawater systems (Epifanio et al. 1973). Although basin culture is used to compare an experimental variable with a control condition, results may be affected by a buildup of molluscan and algal metabolic products which vary with the food supply. Additionally, the pH and oxygen profiles of the seawater column will vary with time affecting the state of algal suspension and aggregation, thus, the uptake of food. In tray culture, a considerable amount of debris is accumulated which encourages growth of competing populations, fouling organisms, and potentially toxic microbes (Michael and Chew 1976), all of which are likely to interfere with test results. The closed-cycle system offers strict environmental control and some economic advantage, but problems reside in evaporation, and in the potential for concentrating toxic organics (Carmignani and Bennett 1976a, 1976b; Mueller and Bradley 1980) and inorganic ions (Epifanio and Srna 1975).

The culture chamber described in this report was designed to meet the following goals: to use "clean" seawater, to accommodate mollusks of different sizes, to remove waste

material continually, to allow for a flexible feeding regime, to provide for simultaneous testing of experimental variables, to be inexpensive and simple to operate on a small scale in laboratory studies but also to have the potential for being scaled-up to a commercially useful size and, finally, to minimize maintenance procedures for rearing mollusks.

### MATERIALS AND METHODS

A diagrammatic view of the entire system is shown in Figure 1, and an exploded view of the culture chamber in Figure 2. Each culture chamber was constructed from a 38.1-cm length of polyvinylchloride (PVC) pipe of 20.3-cm diameter and 1.3-cm wall thickness. The ends of the pipe were sealed with PVC disks cemented in place with a fiberglass resin adhesive, and the cylinder was bisected longitudinally. Holes were drilled to accommodate a 1.3-cm PVC 90° elbow pipe and three PVC or hard rubber stop-cocks which were cemented in place. The PVC tubing was connected to the elbow and cut to a length that corresponded to the top of the chamber. Weather stripping (closed-cell foam tape) was applied to the joining surfaces of the upper and lower halves of the chamber as a sealant.

One-piece frames for culture chamber screens were cut from 1.3-cm thick sheets of PVC. Monofilament nylon screening, Nitex 950 mesh (Tetko, Elmsford, NY)\*, was stretched tightly across the frame and secured with fiberglass resin adhesive. Two size 7 (102 to 178 mm) worm gear clamps secured the support screen between the chamber halves. A pinewood support was constructed for each chamber.

Seawater from Long Island Sound (salinity 27 to 28 ppt) was filtered through polypropylene cartridges with mean retentions of 50  $\mu\text{m}$ , 10  $\mu\text{m}$ , and 1  $\mu\text{m}$ , UV-sterilized, and heated to 26°C in a fiberglass tank. Through a number of joints and sections of PVC tubing, seawater flowed to a manifold and, finally, into the chambers through amber

\*NOTE: Mention of commercial names does not constitute endorsement of products by the National Marine Fisheries Service.

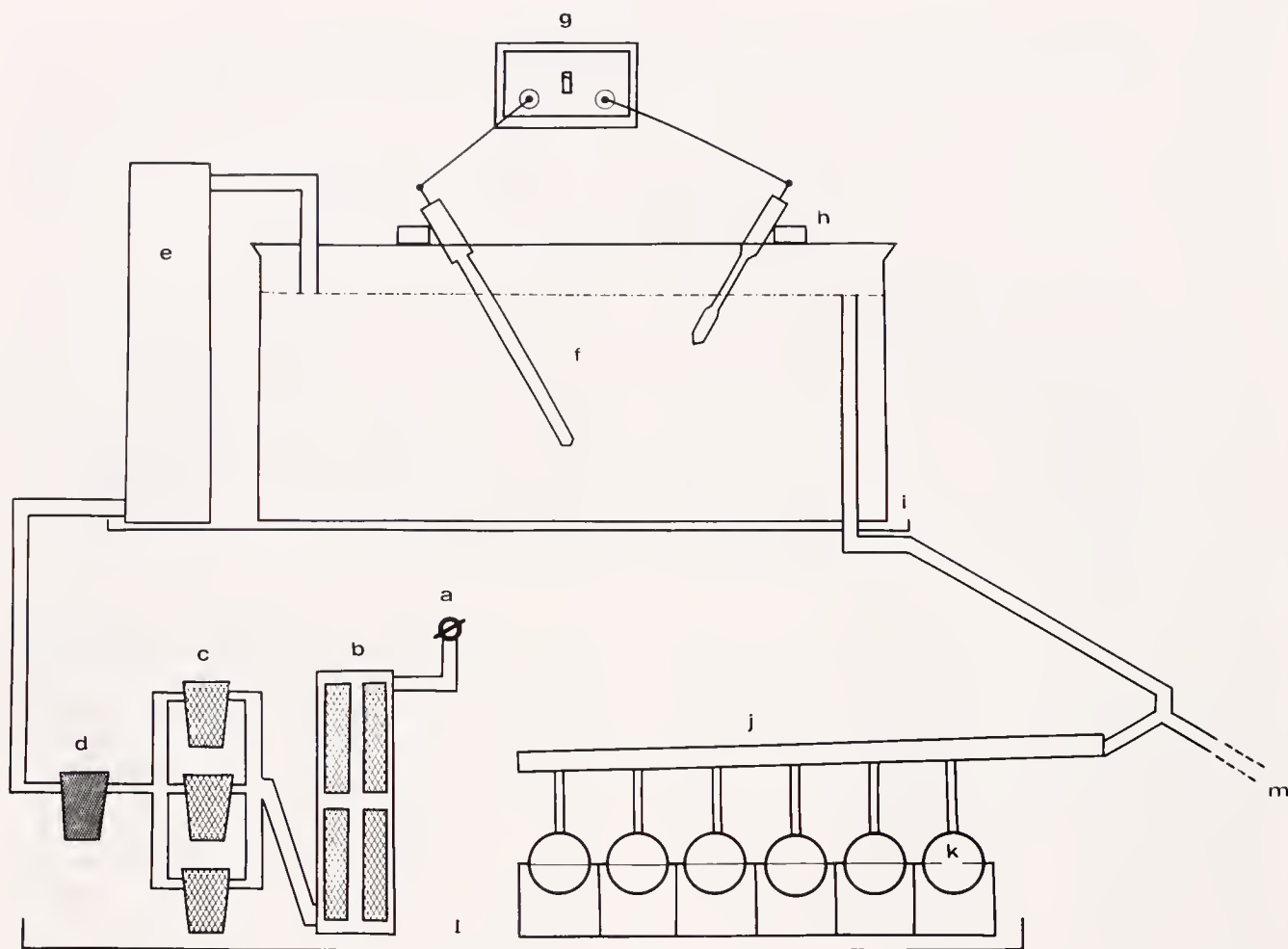


Figure 1. Diagram of seawater supply and arrangement of experimental rearing chambers: (a) seawater tap; (b) polypropylene filters (Industrial Filters, Burlington, MA), 50- $\mu$ m retention; (c) polypropylene filters, 10- $\mu$ m retention; (d) polypropylene filter, 1- $\mu$ m retention; (e) ultraviolet sterilizer (Aquafine Corp., Burbank, CA); (f) fiberglass tank (91.4  $\times$  61.0  $\times$  35.6 cm) with standpipes; (g) temperature control system consisting of 1,000-W immersion heater (Vycor brand glass) and merc-to-merc thermoregulator controlled by electronic relay; (h) wooden supports for immersion heater and thermoregulator; (i) shelf supporting seawater tank; (j) manifold (PVC pipe fitted with hard rubber stopcocks); (k) rearing chamber on pinewood support; (l) water table with drain; and (m) tubing leading to second manifold.

latex tubing. The rate of seawater flow into each chamber was adjusted with the seawater inflow valve to about 800 ml/min, yielding a 6- to 10-minute turnover of the 6.3 l in the chamber. As seawater flowed through the chamber, oysters on the screen were continuously washed in "clean" seawater; this flow tended to carry fecal material and unused food through the screen and out the side arm.

The following species of algae, harvested from a semi-continuous culture system (Ukeles 1973), were tested as foods: *Dunaliella euchlora* Lerche (origin unknown; strain identified by R. A. Lewin but referred to as *D. tertiolecta* by McLachlan 1960); *Tetraselmis maculata* Butcher (Institute of Marine Resources, La Jolla, CA); *Phaeodactylum tricornutum* Bohlin (Plymouth Laboratory culture); *Chlorella autotrophica* Shihira and Krauss (referred to as #580 in culture collections; species isolated at Milford Laboratory); and *Thalassiosira pseudonana* 3H Hasle et Helmdale (Guillard

collection: *Cyclotella nana* Hustedt, Guillard and Ryther 1962). These strains have been maintained in axenic culture for many years in the collection at the National Marine Fisheries Service (NMFS) Milford Laboratory.

Each chamber containing a preweighed group of 50 hatchery-reared seed oysters received daily aliquots of algae equivalent to 500 ml at a density of 0.012 packed cells/10 ml of algal suspension as determined by centrifugation in modified Hopkins tubes (Ukeles 1973). The feeding of equivalent packed cell volumes (PCV) provided oysters with the same total cytoplasmic mass in each algal diet. The number of cells per milliliter in each chamber immediately after adding the algal suspension were: *T. maculata*,  $3.5 \times 10^5$ ; *T. pseudonana*,  $8.7 \times 10^5$ ; *D. euchlora*,  $4.2 \times 10^5$ ; *P. tricornutum*,  $1.1 \times 10^6$ ; *C. autotrophica*,  $2.8 \times 10^6$ . At the time of feeding, inflow of seawater was stopped, the chamber was emptied of 500 ml, and algae were introduced

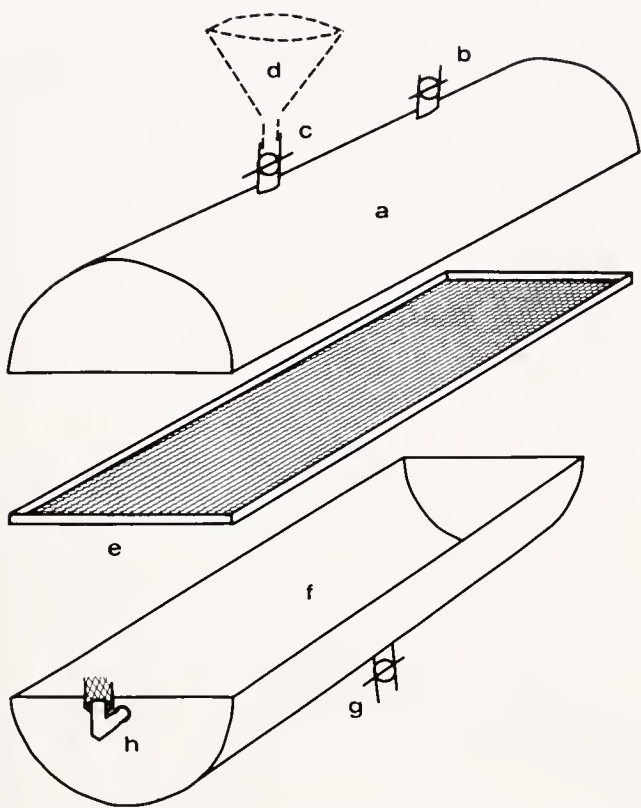


Figure 2. Exploded view of experimental rearing chamber: (a) top half of chamber; (b) seawater inflow valve with stopcock; (c) air vent plus feeding valve with stopcock; (d) feeding funnel supported in nylon-reinforced PVC tubing; (e) nylon monofilament mesh support screen in PVC frame; (f) bottom half of chamber; (g) drain valve with stopcock; and (h) side-arm level. (Pinewood support not shown.)

through the funnel. The oysters were allowed to feed for four hours, after which the seawater flow was resumed until the next feeding. Oysters in the culture chambers were fed daily on weekdays.

The chambers were disassembled and cleaned once a week. At that time, oysters were removed from the screen, cleaned with a soft-bristle brush, gently blotted dry with absorbent paper, and groups of oysters from each chamber were collectively weighed with a Sartorius top-loading balance. Growth response of the oysters to algal diets was evaluated by changes in live weights. The fact that this included shell plus tissue weights was considered acceptable within the context of these experiments.

## RESULTS

The daily routine of feeding and regulating the seawater flow, as well as the weekly routine of cleaning chambers, proceeded smoothly and required little time as compared with basin culture techniques.

Mean live weights of oysters at the start of the experiment were 0.381 to 0.382 gm. Unfed oysters and those fed

with *C. autotrophica* grew slowly for the first 4 weeks at which time their mean whole live weights were 0.475 and 0.477 gm, respectively. Thereafter, no appreciable growth occurred in either group of oysters. Animals fed *P. tricornutum* increased steadily to a mean weight of 0.632 gm, whereas *D. euchlora* stimulated growth to 0.730 gm/oyster in 13 weeks. The greatest increases in size were observed in oysters fed *T. maculata* and *T. pseudonana*; mean weights at 13 weeks were 0.920 and 0.829 gm, respectively (Figure 3). The slight reduction in rate of growth, during the last 3 weeks, of oysters receiving *T. pseudonana* may have resulted from a deterioration in the *T. pseudonana* culture. Mortality of oysters did not occur, with the exception of those receiving *C. autotrophica*; one dead oyster was removed from the latter group in each of weeks 5 and 11.

## DISCUSSION

Although it has been known for many years that laboratory-reared mollusks utilize unicellular algae as food sources (Ukeles 1971), there still exist many algal species of unknown nutritional value. Questions pertaining to the value of artificial and synthetic foods need to be investigated in controlled environments. One source of difficulty in arriving at coherent information is that investigations have been conducted under widely disparate conditions.

This report describes an experimental molluscan rearing chamber that was adapted to study nutritional requirements and feeding behavior of mollusks at different stages of development. The chamber can also be used for investigating factors that affect molluscan growth, such as concentration of nutrients, rate of feeding, water flow, temperature, and pollutants.

For this study a discontinuous feeding schedule was chosen rather than introducing the food with the water supply during the entire day. Evidence from studies with *Crassostrea gigas* (Thunberg) (Langton and McKay 1974, 1976) indicates that a discontinuous feeding schedule results in greater oyster weight increases than continuous feeding. The washing of oysters in a rapid flow of clean seawater to remove wastes, provide oxygen, and offer ample opportunity for ionic exchange was shown to be necessary for rearing oysters and quahogs by Kerswill (1949) and, thus, provided the basis for our feeding chamber design. The regularity of weight increases and lack of mortality in populations of oysters receiving adequate diets give evidence of the effectiveness of the chambers for rearing oyster spat.

Although rearing techniques differ, results obtained in the present study are generally in agreement with previously reported feeding studies. *Thalassiosira pseudonana* yielded very good growth of oysters in the chamber and was also reported to be beneficial to mixed algal diets for *C. virginica* by Epifanio (1979). In the present study, *T. maculata* supported better growth of juveniles of *C. virginica* than four other algal species. A closely related species, *T. suecica*,

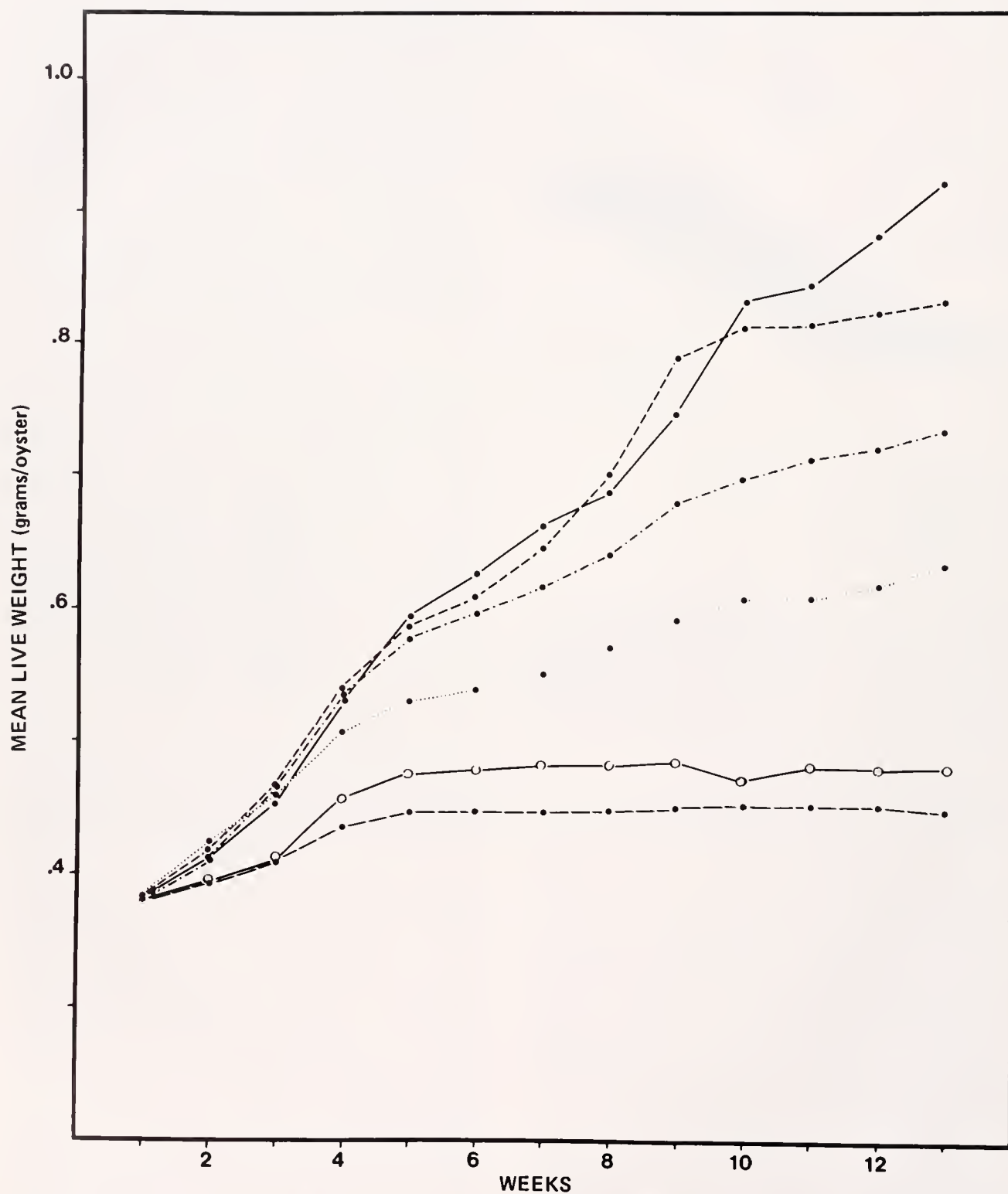


Figure 3. Growth of juvenile oysters reared on algal diets. *Tetraselmis maculata*, solid line solid circle; *Thalassiosira pseudonana*, dash line; *Dunaliella euchlora*, dot-dash line; *Phaeodactylum tricornutum*, dotted line; *Chlorella autotrophica*, diagonal dash line; not fed, solid line open circles.

has been found to be a very good food for juveniles of *Ostrea edulis* Linnaeus (Walne 1970). Results of feeding with *P. tricornutum*, shown in the present study to be a poor or mediocre food, corroborated the findings of Epifanio and Mootz (1975) with *C. virginica* and of Walne (1970) with juveniles of *O. edulis*. *Chlorella autotrophica* was found to be indigestible by larvae of *C. virginica* and, when fed, yielded no larval growth (Babinchak and Ukeles 1979); no growth was observed in this study when *C. autotrophica* was fed to juvenile oysters. In fact, there was some evidence of toxicity. A small increase in oyster growth was observed in the unfed group of animals which

were probably benefiting from stored nutritive material; however, the presence of *C. autotrophica* was sufficient to inhibit this initial growth increase.

We anticipate that, with carefully controlled experiments and use of a standardized rearing technique, some of the more subtle factors of nutrient uptake and utilization may become unraveled.

#### ACKNOWLEDGMENTS

We thank Kenneth Provost for mechanical work on construction of the chambers, and Joseph Twarog and Jane Armstrong for technical assistance.

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## BIOFOULING RESISTANT SHELLFISH TRAYS<sup>1</sup>

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**ABSTRACT** Biofouling of shellfish trays, while highly dependent on site and season, has in many situations either completely prohibited the use of trays or required considerable labor for tray cleaning. The use of 90-10 copper-nickel (Cu-Ni) alloy expanded metal mesh on the critical screening parts of trays has been shown, under normal operating conditions, to be effective in preventing biofouling of the mesh. In addition it has been demonstrated to be harmless to the shellfish, practical in operations, and acceptable to culturists. Since 1977, more than 100 test trays of at least 11 different designs, using 90-10 Cu-Ni expanded metal mesh, have been used by more than 25 commercial, hatchery, and research bivalve culturists in seven countries. Field experiences to date indicate that the use of 90-10 Cu-Ni mesh in shellfish trays improves shellfish growth through better water circulation and substantially reduces labor requirements.

### INTRODUCTION

Before concentrating on the main focus of this paper, it is necessary to review the general status of tray culture, the problems involved, and the variety of equipment currently used in the tray culture of bivalve mollusks. Off-bottom culturing of shellfish in trays is widespread, from oysters in Europe (Meixner 1976), to pearl oysters (Mizumoto 1976) and scallops (Sanders 1973) in Japan. Commercial tray culture of oysters and hard clams, and tests with scallops are also common in the United States, for both hatchery and grow-out applications, although no comprehensive survey paper is currently available. Because most United States operations are relatively small, it is difficult to determine the magnitude of the tray-culture effort, but it certainly represents only a small part of the total shellfish industry. Because of the capital expense of tray systems and the labor involved with tray culture, such a technique is limited to the higher priced segments of the shellfish markets or to hatchery operations. Superior quality of the product sometimes compensates for the additional costs.

Several types of shellfish trays are commercially available in the United States. These trays are made with galvanized hardware cloth, vinyl-dipped hardware cloth, or plastics. For hatchery use, fine meshes are often inserted on larger mesh grow-out trays. In addition, trays primarily intended for other purposes, such as bread trays or trays for oyster species, have been modified with deeper solid bottoms to hold a substrate (mud, sand, or gravel) for use in clam-hatchery and nursery operations. A number of tray systems, involving considerable similarities in requirements, equipment, and management techniques, are also being developed for culturing lobsters (Van Olst et al. 1977), and much of that experience is useful for bivalve systems. Most bivalve shellfish trays are a meter or less in horizontal dimensions, and 7 to 10 cm in height, if no substrate is required, and are usually rectangular, square, or round. Some are designed

primarily for horizontal flow through the trays, for use in natural areas and in raceways, and others for flow primarily in the vertical direction for use in towers or baffled raceways. Most trays are of about the same size and hold approximately 0.009 to 0.026 m<sup>3</sup> (¼–¾ bu) of fully grown shellfish. Some trays can be stacked and assembled into convenient modules of up to a dozen trays. These modules have been floated, rafted, long lined, and placed into frames which hold them off the bottom. Other variations include Japanese lantern nets, rectangular trays made of treated or coated weldwire mesh, and large cylindrical trays of several designs with about 10 shelves wrapped with a single piece of removable mesh. The cylindrical type is currently popular in Europe. Because many culturists also make their own trays or adapt available food industry containers, the variations in design are numerous.

A major problem preventing greater use of trays is exemplified by one of the most widely used commercial trays. This tray is a square, injection-molded polypropylene unit about 60 × 60 × 5 cm (23 × 23 × 2 in.). The tray's critical sides are polypropylene with 0.6 cm (0.25 in.) holes rather than a mesh. The sides have only a small open area and readily biofoul, resulting in loss of water circulation and subsequent sharp reductions in shellfish growth (Michael and Chew 1976). Water circulation through trays is essential for bringing in food as well as oxygenated water to the shellfish. Biofouling of meshes can significantly affect tray-culture economics (Glenn and Aguilar 1981). The problem is not unique to this particular design.

There are two biofouling factors which reduce water flow. One is the biofouling of the meshes through which water enters and leaves the trays. The other is the biofouling and siltation within the trays themselves. This latter factor is particularly important when the primary flow through the tray is horizontal and the tray is shallow. Some culturists with nested trays have found that to get adequate shellfish growth they have to use the plastic trays singly. This allows some additional water flow in the vertical direction but negates the handling advantages of being able to stack trays.

<sup>1</sup>Research on the use of copper alloys for shellfish trays has been supported by the International Copper Research Association, Inc.

An alternative is to place spacers between each tray in a stack. The desire to increase the effective flow area is the reason some shellfish facilities are designed to have primarily a vertical flow direction through the trays. Biofouling is a very serious problem in some localities and many of the trays purchased or made by culturists in the past are no longer used because of this problem. Biofouling is very specific with respect to site, depth, and season; wide variations in intensity being common, even within relatively small geographic areas. Mesh dimensions and materials can also strongly influence the degree of biofouling; some types of mesh are much more susceptible than others (Huguenin and Ansuini 1981).

Biofouling can be combated through two methods: prevention and control. In this paper we shall examine biofouling prevention through the judicious use of materials that are resistant to biofouling. Although not specifically intended for use with trays, a large number of other techniques are commonly used in shellfish culture for prevention and control of biofouling (Arakawa 1973). Unfortunately, in tray culture frequent use of brute-force cleaning methods are very common. The costs of that approach can be prohibitive. Biological control of biofouling and siltation in trays via small crabs has been found to be very effective under some conditions (Hidu et al. 1981). Such biological control methods have considerable potential for solving the practical problem of biofouling and siltation either in combination with special materials or as an alternative.

The idea of using the considerable inherent biofouling resistance of copper alloys for application to marine aquaculture and seawater screening was proposed by Huguenin and Ansuini (1975). The idea has been successfully pursued in the design of shellfish trays, marine fish cages (Huguenin et al. 1979), and seawater intake screening systems (Ansuini et al. 1978). The first shellfish test trays were rectangular 60 × 60 × 8 cm with 13-mm square mesh (0.5 in. nominal square mesh) of 1.6-mm diameter pure copper wire. Control trays of similar dimensions were made of aluminized steel and plastic-coated steel. Those trays were tested during a more comprehensive program which involved controlled indoor tests and copper uptake studies with American oysters (*Crassostrea virginica*) (Hammar 1976). The objective of those tests was to examine the compatibility of copper materials in shellfish culture applications. The results from controlled indoor experiments indicated that copper additions to the water at or below existing background levels did not result in any significant increase in bioconcentrations of copper (Hammar 1976).

Some problems arose with the copper trays that were deployed in estuarine environments. Those trays were made of pure copper and the nodes of woven mesh were not fixed; therefore, the trays were very pliable, subject to fretting at the nodes, and lacked structural strength (Hammar 1976). Because copper is very soft, the trays were

also subject to erosion and physical damage. The corrosion rate of pure copper is an order of magnitude or greater than that of some copper-nickel alloys (90-10 and 70-30 Cu-Ni), which also exhibit fouling resistance. The oysters in the pure copper wire trays at one test site exhibited high concentrations of copper in the tissues because of the high corrosion rate of copper and the nearly stagnant water conditions at that site (Hammar 1976).

Some copper-nickel alloys have a long and successful history of use in the marine environment (Hunt and Bellware 1967). Major advantages of the alloys include low and uniform corrosion rates, and fouling-resistant properties, which translates into long service lives. However, to be biofouling resistant, copper alloys must be allowed to freely corrode. The minimum release rate of copper ions into the environment to maintain fouling resistance has been estimated to be 5 mg/100 cm<sup>2</sup>/day (LaQue 1972). In uniformly corroding copper alloys, that would correspond to a corrosion rate of approximately 20 μm/yr (0.008 in./yr or 0.8 mil/yr). This value has long been accepted as the minimum required to maintain a toxic concentration of copper ions at the metal-seawater interface. However, recent work has shown that copper-nickel alloys retain their fouling resistance even when corroding at only 1.3 to 2.5 μm/yr (0.05 to 0.1 mil/yr), well below the rate necessary to maintain a toxic level of copper ions at the metal-seawater interface. This implies that the complex corrosion product film which forms on these alloys is of itself toxic (Efrid 1975). This would explain the inability of copper materials to protect adjacent noncopper parts from fouling. In addition, the fouling protection, while substantial, is not always 100%. The 90-10 Cu-Ni mesh can become fouled by strong blooms, individual fouling organisms occasionally do set, and there is usually some residual biofouling (Huguenin and Ansuini 1981).

#### MATERIALS AND METHODS

Prior work with copper and oysters, including the pure copper trays previously described, was sufficiently promising that additional efforts were initiated in 1977. The basic objective was to develop practical shellfish culture systems using biofouling-resistant copper alloy materials advantageously. The research and development program included modification and distribution of commercially available trays to a number of organizations for testing, and distribution of test quantities of copper alloy materials to others. Many groups, here and abroad, were involved (Table 1). Some efforts were externally funded and others were privately supported. In the latter category were a number of tray-user requirements/marketing studies and tray-system design/costing studies.

Copper additions to the water were based on an exposed surface area flow rate, and corrosion rate relationship (Huguenin and Ansuini 1975). By replacing only the

TABLE 1.  
Tests of shellfish trays using 90-10 Cu-Ni expanded metal mesh.

Country	Items Supplied	Number of Testing Groups	Species (not complete)	Comments
United States	Modified trays and Cu-Ni materials	8	<i>Mercenaria mercenaria</i> <i>Crassostrea virginica</i> <i>Argopecten irradians</i> <i>Ostrea edulis</i>	At least three different tray designs.
Canada	Modified trays and Cu-Ni materials	2	<i>Ostrea edulis</i> <i>Crassostrea virginica</i>	Three different designs, two are modifications of commercial trays.
England/Scotland	Modified trays and Cu-Ni materials	8	<i>Pecten maximus</i> <i>Chlamys opercularis</i> <i>Crassostrea gigas</i> <i>Ostrea edulis</i>	Six designs of trays involved.
Northern Ireland	Modified trays	1	<i>Crassostrea gigas</i>	Also testing UK design.
Spain	Modified trays and Cu-Ni materials	2	Oysters (sp. unknown)	Several designs, including one 800-tray test program.
New Zealand	Modified trays	1	<i>Ostrea lutaria</i> <i>Saccostrea glomerata</i> <i>Mytilus edulis</i>	
Chile	Modified trays	1	<i>Crassostrea gigas</i>	
France		2	<i>Ostrea edulis</i> Scallops (sp. unknown)	Single design.

critical sides of trays where water flow is essential and by the use of a low corrosion rate alloy, such as 90-10 Cu-Ni (CA-706) rather than pure copper, the situation can be greatly improved. The alloy is much stronger and the copper additions to the water under normal field conditions are miniscule and not a potential biohazard to the culture animals. If one replaces the critical sides of the previously mentioned polypropylene trays with 1 cm (3/8 in.) nominal (this is a designation and not an exact dimension) 90-10 Cu-Ni expanded metal mesh, those composite trays have a minimum acceptable water velocity of about 1.0 mm/s (0.002 knot) through the trays. This value can be calculated by using a stabilized corrosion rate of 2.5  $\mu\text{m/yr}$  (0.0001 in./yr), a density of 9 g/cm<sup>3</sup> (0.323 lb/in.<sup>3</sup>), an addition of 2 ppb of Cu-Ni to the water, a surface area of 167 cm<sup>2</sup> (0.18 ft<sup>2</sup>) for all metallic surfaces on one side of such a tray, and an unobstructed area of 140 cm<sup>2</sup> (0.15 ft<sup>2</sup>) through one side of the tray. This is an extremely low water velocity compared to circulation normally encountered in coastal and estuarine areas. Because stagnant areas are not suitable for growing shellfish, these trays do not add a water velocity constraint which does not already exist for other reasons. The same technique can also be used to carry out calculations for other sets of conditions.

Some limitations should be noted when using Cu-Ni trays. The initial corrosion rate, while the oxides are forming, may be an order of magnitude higher than the stabilized

value. When new, trays should be leached in running seawater for at least a week before use. This also protects against undesirable initial releases from the plastics and other materials. In addition, care should be taken to ensure that there are no other metals in direct contact with the mesh to eliminate any possibilities of electrolysis. Care should also be exercised if the trays with shellfish are to be placed, even temporarily, in any sort of tanks. Even though the pumped flow rate may be considered high, pumped flow is likely to be miniscule compared to natural circulation in an estuary.

More than 40 polypropylene shellfish trays were modified over a period of several years using 1 cm (3/8 in.) nominal 90-10 Cu-Ni expanded metal mesh on the side panels. The 90-10 Cu-Ni mesh used in this modification, as well as in all other trays described in this paper, has a gauge of 0.9 mm (0.035 in.), strand width of 1.3 mm (0.050 in.), 76% open area 20° to vertical, and holes of about 0.9 × 1.5 cm (0.35 × 0.6 in.). The mesh in the modified trays was secured with 90-10 Cu-Ni wire and the edges were melted into the polypropylene with a heat gun. These trays were distributed, starting in August 1977, to a number of users representing a considerable range of operating environments and culture species for comparative evaluation against unmodified trays (Table 1). In all cases similar control trays with conventional mesh were either available or were supplied. Field test conditions covered the range from hatchery,

nursery, and grow-out operations to the maintenance of brood stocks and a wide variety of shellfish species. The tests were designed to determine productivity increases resulting from better water circulation, practicality, maintenance, possible design improvements, and possible problem areas. A number of organizations were supplied with 90-10 Cu-Ni expanded metal mesh and other Cu-Ni materials to enable them to modify some of their own shellfish trays. Some of those are of unique design. They are also included in Table 1. As a consequence, some commercially available circular polystyrene shellfish trays, about 46 cm (18 in.) in diameter and 5 cm (2 in.) deep, have also been modified and tested. To date, shellfish trays of 11 different designs have been built or modified using Cu-Ni mesh. All of the organizations are either involved with shellfish culture, shellfish research and development, or are commercial shellfish culturists.

### RESULTS AND DISCUSSION

Feedback on field experiences with several types of modified trays has been received from most organizations that received such trays or modified their own. This feedback ranged from high quality scientific data and qualitative judgments to "no results, equipment lost at sea." More data are still being collected. The most important results to date have been the universal acceptance by the participants of Cu-Ni materials as compatible with shellfish culture and their recognition of the fouling resistance of these materials. In addition, no detrimental effects on any of the bivalves, due to use of copper alloys, have been observed by any of the testing participants. Shellfish growth and survival in the modified trays have been, in all cases, equal to or better than that in the control trays. In some cases growth and survival were based on subjective judgments of culturists or on only a few rudimentary measurements; in other cases careful experimental design and measurements were carried out. In one series of comparative tests with oysters in Canada, not only was the growth in the modified 90-10 Cu-Ni trays about 50% greater than that in controls but the standard deviations in size were about 30% less than that of the controls. The implications from this and other tests are that improved water circulation not only improved growth but produced more uniformly sized animals. Most of the concerns and doubts of the participants involved the future costs of such trays and the merits of tray culture, *per se*, in comparison to other shellfish culture methods.

Tray culture involves a relatively high initial expenditure for shellfish trays and constant attention is generally required, as exemplified by the frequent cleaning practices of some culturists. To date many culturists have handled the modified trays as they would any other trays, placing them in with other trays and using the same cleaning schedules. Therefore, these culturists did not receive all of the benefits inherent in the new materials. Quantitative data on the degree of reduced maintenance produced by

the fouling resistance of Cu-Ni meshes have been acquired in only a few cases. However, all feedback to date clearly indicates the existence of substantially reduced biofouling in actual culture operations. The few quantitative values received from culturists who altered their cleaning schedules because of the Cu-Ni mesh trays indicated savings of 60 to 100% of the labor previously used to maintain the trays.

The modification of commercially available trays to insert a 90-10 Cu-Ni mesh is a laborious process and can be justified only in the context of a research operation or to verify design concepts. Two such plastic tray designs were modified and tested. However, preliminary calculations and discussions indicate that a future tray of improved design and compound construction (90-10 Cu-Ni expanded metal mesh for water circulation, plastic for structure) would cost 10 to 30% more than the same tray with plastic mesh. In this case the loads on the mesh are minimal and the primary requirement for selection of a mesh thickness is to assure a long useful service life. Therefore, the relatively expensive Cu-Ni is used only where it is needed most for fouling control and in thin gauges.

A 1981 survey of six commercially available and utilized shellfish trays in the United States indicated that purchase prices vary from \$15.60 to \$29.05/m<sup>2</sup> (\$1.45 to \$2.70/ft<sup>2</sup>) of tray bottom area depending on tray type, materials and purchase quantity. Copper-nickel trays would be on the high side of this range, but this does not take into consideration their substantial scrap metal value not inherent in other materials.

A number of ways exist for combining plastic and Cu-Ni mesh; one of these is injection molding. Unfortunately, this promising approach requires a large launching production order to help recover the substantial initial expenses involved with mold design and fabrication. In addition, once the mold has been made, it is very difficult to change tray dimensions or other tray characteristics.

Some existing tray designs lend themselves to a substitution of materials. Tayside Engineering of Scotland, has designed, tested, and now commercially offers a 90-10 Cu-Ni expanded metal mesh version of their standard wooden tray system. This tray is about 75 × 75 × 6.5 cm (30 × 30 × 3 in.), and a small number are being used in Scotland and a few in Northern Ireland. Additional modifications of standard wooden trays with 90-10 Cu-Ni mesh have been carried out by the University of Maine, Darling Center, with their 61 × 61 × 15-cm (24 × 24 × 6-in.) trays and by Dart Oyster Fisheries Ltd., of the United Kingdom, with their 183 × 81 × 8-cm (72 × 32 × 3-in.) trays. Any tray constructed of an electrically nonconductive structural materials, such as wood or plastic, including home-built trays (Richmond 1974), can usually be readily modified to accept a 90-10 Cu-Ni mesh. If wire or staples are used to secure the mesh, they must be made of the same material or one cathodic to 90-10 Cu-Ni, such as Monel. Many common materials, such as steel, are unsuitable. In

addition, there is some interest in using a single, removable, wrap-around sheet of Cu-Ni mesh with a large cylindrical, 10-shelf, plastic tray system. The danger with this approach is in assuring that the various materials used, especially any fasteners or staples, are compatible with the 90-10 Cu-Ni alloy.

Another approach would be to construct a tray entirely of Cu-Ni mesh. Mariculture Ltd., England, made a small number of 64 × 58 × 8-cm (25 × 23 × 3-in.) oyster trays by folding up the mesh and securing the corners. These trays have been in service without any problems since March 1980. Devon Oysters Ltd., England, also followed that approach with two different designs currently under test. The limitations of that approach involve problems of nesting multiple trays, possible structural problems if thin gauges are used, joining of materials especially in corners, and the possibly unnecessary and excessive use of a relatively expensive mesh material. A variation on that approach is the 28-cm (11-in.) diameter by 8.5-cm (3.3-in.) deep cylindrical trays made by Trefimetaux of France. Those trays nest readily, are reinforced with solid strips of 90-10 Cu-Ni material, and are similar in design to an available Spanish plastic tray. Trefimetaux's trays have been sent to several French shellfish culturists for testing and evaluation.

Another approach is being tested by Aquicultura del Atlantico SA of Spain. They built frames constructed of commercially available fiberglass shapes to form shelves which hold twenty 35 × 35 × 7.5-cm (14 × 14 × 3-in.) trays made of 90-10 Cu-Ni expanded metal mesh. One advantage of this approach is that the individual trays do not need much strength and can be made of thin gauge materials because structural support is provided by the frame. The test program involves 800 trays distributed over four sites.

Estimating the world market for shellfish trays is very difficult at best. While pearl culturing in Japan alone uses about 100,000 trays (Mizumoto 1976), the numbers of trays used in other activities and regions are much more modest. A marketing survey on shellfish tray-user requirements was conducted by the authors in 1978, and resulted

in approximately 50 replies from culturists in North America and Europe. In response to a question on the importance of developing a tray with biofouling resistant meshes, 52% responded "extremely valuable," 33%, "important," and only 15% of "minor value." This survey also indicated that the demand for trays capable of holding a substrate was about one half of that for more conventional trays and that the average commercial sale would probably not exceed 1,000 to 2,000 trays. However, individual operations exist with substantially larger needs. Large volume tray users tend to make their own trays. In addition, because of individual user preferences and requirements, no single tray design is likely to cover the total market, although a design family of related trays might come close. The probable shellfish tray market for commercially produced trays in North America and Europe for the next few years is unlikely to exceed 100,000 trays (discounting the fact that some culturists will continue to make their own trays). Market demand, however, may be very sensitive to whether practical solutions can be developed for problems that currently limit tray culture.

## CONCLUSIONS

There are a number of possible approaches and variations in design for use of 90-10 Cu-Ni mesh in shellfish trays. It is clear that such mesh has considerable biofouling resistance and can substantially reduce labor requirements while increasing growth through improved water circulation. It is also clear that Cu-Ni mesh trays are acceptable and promising for a wide variety of field conditions and culture species. With careful design, such trays should be only slightly more expensive than similar trays of more conventional mesh material. They should also provide considerable improvements in performance over conventional systems.

## ACKNOWLEDGMENTS

The authors gratefully acknowledge the assistance of Paul Chanley, Frank Ansuini, Brian Muise, Tony Rayne, and all other participants in the research described in this paper.

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## DISTRIBUTION, REPRODUCTION, AND GROWTH OF MANILA CLAM, *TAPES PHILIPPINARUM* (ADAMS AND REEVES), IN BRITISH COLUMBIA

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**ABSTRACT** Manila clams (*Tapes philippinarum*) were first found in British Columbia in Ladysmith Harbour in 1936. In 30 years they spread throughout the Strait of Georgia and along the entire western coast of Vancouver Island to become one of the major intertidal bivalves. In the 1960's, Manila clams spread to the Queen Charlotte Strait area and, in the 1970's, to the central coastal area. Probable dispersal routes to these areas are discussed. Two intentional transplants to establish breeding populations in the northern part of the Province were not successful. Growth rates of Manila clams are fastest in the Strait of Georgia where water temperatures are highest, and slowest in Queen Charlotte Strait where water temperatures are lowest. Further northward dispersal of this species along the British Columbia coast is discussed.

### INTRODUCTION

Manila clams (*Tapes philippinarum*) are indigenous to Japan between latitudes 25 to 45°N where they are widely used in the commercial fishery (Tamara 1966). The species was accidentally introduced into British Columbia presumably with seed of the Pacific oyster *Crassostrea gigas* (Thunberg) from Japan, and spread rapidly throughout the southern part of the Province (Quayle 1964). Manila clams soon found general acceptance as an edible mollusc in British Columbia and now support commercial and recreational fisheries (Quayle and Bourne 1972) (Figure 1); the accidental introduction is considered beneficial. Discovery and initial dispersal of this species in southern British Columbia were previously reported (Neave 1949, Quayle 1964, Quayle and Bourne 1972, Bourne 1979). The present paper provides recent information on the continuing spread of this bivalve, routes of dispersal, reproduction, and compares growth rates in several areas in British Columbia.



Figure 1. Annual commercial landings of Manila clams (whole weight) in British Columbia, 1951-1980.

### DISPERSAL IN THE STRAIT OF GEORGIA

In British Columbia, Manila clams were first found in Ladysmith Harbour (lat. 49°N) in 1936. They spread rapidly throughout the Strait of Georgia (Quayle 1960, 1964) (Figure 2) and, by 1942, Neave (1949) reported they were the most common bivalve in Departure Bay at Nanaimo about 25 km north of Ladysmith Harbour. In 1941, they had entered the commercial catch (Quayle and Bourne 1972).

Although the Strait of Georgia is north of the traditional latitudes where Manila clams occur in Japan, water temperatures in the Strait are favorable for Manila clam reproduction. Holland and Chew (1974) and Ohba (1959) reported the presence of ripe gonads and subsequent spawning in Manila clams when water temperatures exceeded 14°C. Mann (1979) found gonadal development slow at 12°C and no spawning until temperatures reached 15°C. In the Strait, monthly mean surface water temperatures of 15°C and above are common during summer months (Hollister and Sandes 1972), which is adequate for successful reproduction.

Surface currents aided the spread of Manila clams in the Strait of Georgia because water circulation is open and no natural barriers are present to prevent spread of pelagic larvae. Waldichuk and Tabata (1955) and Waldichuk (1957) showed there is a general counterclockwise surface circulation in the Strait. Tully and Dodimead (1957) described the dominant feature of its tidal circulation. On flood tides, water from the south is displaced northward, principally on the mainland side. The flood tide from the north is strongest on the Vancouver side and creates a southward flow. Thus, there appears to be a continuity of flow northward along the mainland side of the Strait, across the northern end and then southward along the Vancouver Island side. On ebb tides, the southward movement is negligible on the mainland side, but it is the dominant flow along the Vancouver Island side of the Strait. Such currents, along with wind-driven currents, would disperse pelagic larvae throughout the Strait.

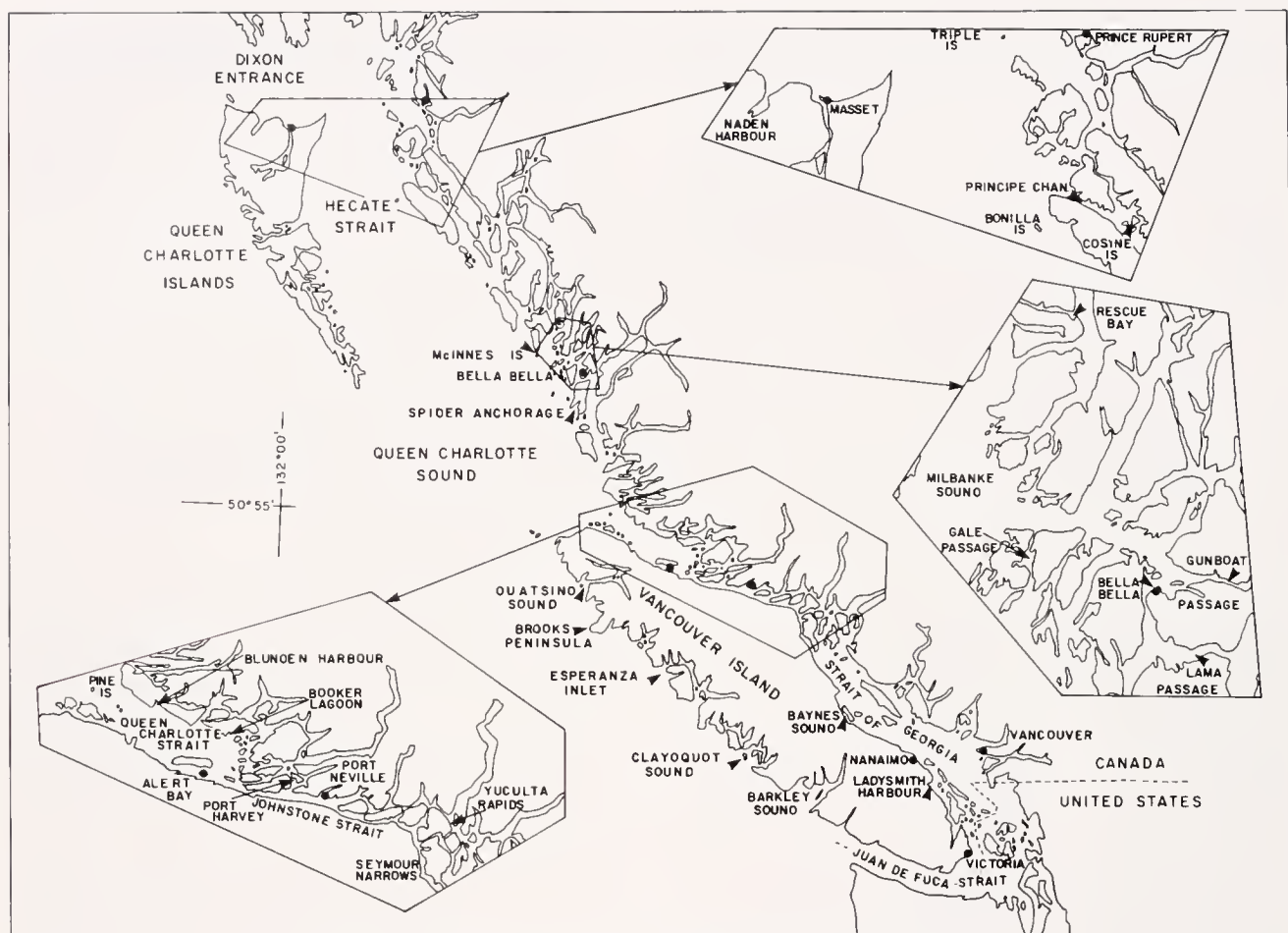


Figure 2. Map of British Columbia showing locations mentioned in the text.

Manila clams inhabit the mid-to-upper portion of the intertidal beach, an ecological niche that apparently was not dominated by any species prior to its introduction, and this contributed to the rapid spread of the species in the Strait. Maximum tidal amplitude in the Strait is about 4.9 m. Width of the Manila clam zone varies with slope of the beach; on steep-sloped beaches it may be only a few meters, and on a few wide beaches with gentle slopes, the width is about 75 m. Manila clams do not occur in the sub-tidal area in British Columbia. Heavy mortalities occasionally occur in abnormally cold winters (e.g., the winter of 1968–1969 [Bourne, unpublished]) because the clams occupy upper beach levels and burrow to only shallow depths in the sediment (10 cm maximum).

On the Pacific coast of North America, Manila clams were first found in British Columbia rather than in the State of Washington where earlier and heavier plantings of Pacific oyster seed from Japan occurred. Their presence was not recorded in Washington until the early 1940's. Manila clams are now abundant in Puget Sound and are used in both the commercial and recreational fisheries (Goodwin 1973, Williams 1978).

Surface water currents from Puget Sound do enter the southern part of the Strait of Georgia and could carry pelagic larvae northward. The introduction and spread of Manila clams in British Columbia undoubtedly resulted from stock planted there and not from dispersal of clams in the Puget Sound area. Similarly it is unlikely there was a southward movement of Manila clams from British Columbia to Puget Sound; the present stock in Puget Sound undoubtedly developed from clams imported there.

Manila clams did not spread northward of the Strait of Georgia because of the cold water temperature barrier at Seymour Narrows and the Yuculta Rapids (Figure 2). Although monthly mean surface water temperatures of 14.3°C have been recorded at the southern end of Seymour Narrows (Hollister and Sandes 1972), water temperatures there and to the north are probably not sufficient to allow spawning or larval development. Water temperatures at the Narrows are probably colder than at the southern end; a 1-year observation at Yuculta Rapids showed a maximum monthly mean surface water temperature of 9.9°C in July (Hollister and Sandes 1972). No Manila clams have been found during extensive surveys of beaches immediately

north of this area (Bourne, unpublished). Commercial landings have been reported from some northern areas in the *Fisheries Statistics*, but these are believed to be in error.

Dispersal of Manila clams southward, through Juan de Fuca Strait, did not occur, probably because of the cold water barrier. Maximum monthly mean water temperatures at three sampling stations in this area are all below 12°C (Hollister and Sandes 1972), too low to allow spawning and larval development.

#### DISPERSAL ALONG THE WESTERN COAST OF VANCOUVER ISLAND

Manila clams occur along the entire western coast of Vancouver Island but dispersal there is less well documented than in the Strait of Georgia. They were probably introduced when Pacific seed oysters were planted in Barkley Sound (Figure 2); the exact date of the introduction is unknown but Manila clams were abundant there in the early 1950's (D. B. Quayle, Pacific Biological Station, personal communication). Water temperatures in Barkley Sound are suitable for Manila clam reproduction and the species spread rapidly throughout the Sound, again occupying the mid-to-upper portion of intertidal beaches.

Dispersal along the western coast of Vancouver Island was rapid and, by the late 1950's, Manila clams were established in Esperanza Inlet (Quayle 1960) about two-thirds northward along the western coast of the Island (Figure 2).

Inshore currents along this coast have a northward direction (Tully 1937) and would carry larvae that drifted out of sounds northward along the coast. Monthly mean summer water temperatures along the outer coast range from 13 to 16°C (Hollister and Sandes 1972) which is sufficient to permit larval development, particularly in summers with above-average water temperatures. Yoshida (1953) stated that the larval period of Manila clams at these temperatures would be 3 to 4 weeks and Williams (1978) reported a similar period in Puget Sound. In a 3- to 4-week period, larvae could drift out of a sound, be carried northward along the coast, enter another sound, settle, and establish a population. Water temperatures in local areas in sounds along the western coast of the Island are warmer than on the outer coast and are quite adequate for successful reproduction. Manila clam populations in inlets along the western coast of Vancouver Island occupy the mid-to-upper part of the intertidal beach.

It was expected that the spread of Manila clams might be halted at the Brooks Peninsula because it tends to be a biological barrier. Species such as the clam *Rhamphidonta retifera* Bernard occur south but not north of this peninsula (F. R. Bernard, Pacific Biological Station, personal communication). However, in 1966, an extensive population of clams was found in Quatsino Sound, the most northerly major inlet on the western coast of Vancouver Island (Figure 2).

In 30 years, Manila clams spread throughout the Strait

of Georgia and along the western coast of Vancouver Island to become one of the major intertidal bivalves.

#### INTENTIONAL TRANSPLANTS

Two attempts were made to transplant populations of Manila clams to the northern coast of British Columbia (Figure 2). In 1962, about 15,000 adults from Ladysmith Harbour were planted in Naden Harbour and a similar number were planted in Masset Inlet on the northern coast of the Queen Charlotte Islands (D. B. Quayle, personal communication) (Figure 2). In 1969, about 100 adults from Baynes Sound in the Strait of Georgia were introduced to Cosine Island, Principe Channel.

Clams survived in both areas but growth was poor, no apparent reproduction occurred, and the populations eventually disappeared. The reasons these populations did not become established are unknown, but low water temperatures were probably a major factor. Maximum monthly mean surface water temperatures of slightly above 14°C have been recorded at Masset (Hollister and Sandes 1972); Black and Elsey (1948) found surface water temperatures were generally below 14°C at the head of the Inlet (where the transplant occurred) although they have risen to 20°C briefly in August. No water temperature data are available from Cosine Island but nearby at Bonilla and Triple islands, monthly mean water temperatures rarely go above 13°C. The time-temperature period for gametogenesis, as described for *C. gigas* and *Ostrea edulis* Linné (Mann 1979), may have been too short at both localities to permit complete gonadal development. If the Naden Harbour population had spawned the larvae may have been flushed out into Dixon Entrance where low water temperatures could cause mortalities. The size of the introduction at Cosine Island may have been too small to permit establishment of a population. If successful breeding had occurred at either location, juveniles may have been killed because of low winter temperatures.

#### DISPERSAL TO OTHER AREAS

In 1972, two adult Manila clams (47- and 48-mm shell length) were found at Spider Anchorage in the central coastal area of the Province (lat. 51°51'N; long. 128°13'W) (Figure 2). Eleven more clams were found there in 1973, ranging in length and age from 45 to 60 mm and 7 to 9 years, respectively, and apparently arrived there in the mid-1960's (Bourne, unpublished). All clams were at the mid-intertidal beach position. Further extensive surveys of this area and areas to the north of it in 1973 failed to yield any more Manila clams.

Extensive surveys in the Queen Charlotte Strait-Alert Bay area (Figure 2) in the 1960's and early 1970's did not produce any Manila clams. In 1979, 12 live and 32 dead clams were found in three isolated locations in this area (Port Harvey, Fife Sound, and Booker Lagoon). Live clams ranged in length from 31 to 55 mm and in age from 5 to 10 years.

Manila clams probably arrived in the Queen Charlotte Strait-Alert Bay area in the late 1960's and again occupied the mid-portion of the intertidal beach. Populations are scattered and sparse, and apparently no recent reproduction or reintroductions have occurred. Whether the population is large enough to establish a reproducing population or if continuing reintroduction of larvae must occur to maintain it, is unknown.

In 1980, a survey was made of beaches around Bella Bella in the central coastal area; Manila clams were found in substantial numbers on 14 of 46 beaches surveyed, again occupying the mid-to-upper portion of the intertidal beach. Densities ranged from 1 to 162 clams/m<sup>2</sup> with the highest occurring in Milbanke Sound and Gunboat Passage. Populations were found on beaches close to the eastern edge of Hecate Strait and also on beaches well into some of the passages where residual surface current flow is outward into Hecate Strait. Shell lengths ranged from 11 to 56 mm and most clams were smaller than the commercial legal size limit of 38-mm shell length (Figure 3). Some samples had more than one year-class indicating the population is either consistently being supplied with larvae from other sources or, more likely, that local reproduction has occurred.

Age analysis (see below) indicates the large dominant year-class of clams at Gunboat Passage is approaching 4 years of age. The two year-classes in Milbanke Sound are approaching 2 and 4 years of age and those in Gale Passage are 2 to 4 years in age.

In 1981, another survey was carried out in the northern area, Principe Channel to Prince Rupert (lat. 53°10'N to 54°20'N), but no Manila clams were found.

The most northerly record of Manila clams in British Columbia is Rescue Bay, Mathieson Channel (lat. 52°30'N).

#### DISPERSAL ROUTES

The few clams found at Spider Anchorage in 1972 and 1973 were probably the result of settlement of larvae from a spawning in the Quatsino Sound area that drifted across Queen Charlotte Sound (Figure 2). Water temperatures in the Quatsino Sound area, as recorded at Kains Island at the entrance to the Sound (Hollister and Sandes 1972), are sufficient to permit gametogenesis and spawning, particularly in years of above-average water temperatures which occurred in 1957, 1958, 1963, and 1967 (A. J. Dodimead, Pacific Biological Station, personal communication). Evidence in support of the transport of larvae across Queen Charlotte Sound is available from an examination of the zonal component of the Ekman transport (wind-driven surface flow). This transport is generally offshore during May to September off northern Vancouver Island but there are years in which the net surface transport is onshore and relatively high in August and September, at a time when sea surface temperatures are usually favorable for larval survival (Dodimead 1980). For a 3- to 4-week larval period (Yoshida 1953, Williams 1978), a net movement of about 4.6 km/day

is required to transport larvae a distance of approximately 120 km across Queen Charlotte Sound. Assuming a mixed-surface layer depth of 5 m, it would require a net onshore Ekman transport of about 270 metric tons (t)/sec/km. Mean monthly values of this magnitude have been reported, particularly in September at latitude 50°N and longitude 130°W (Ballantyne and Wickett 1978, Table 27).

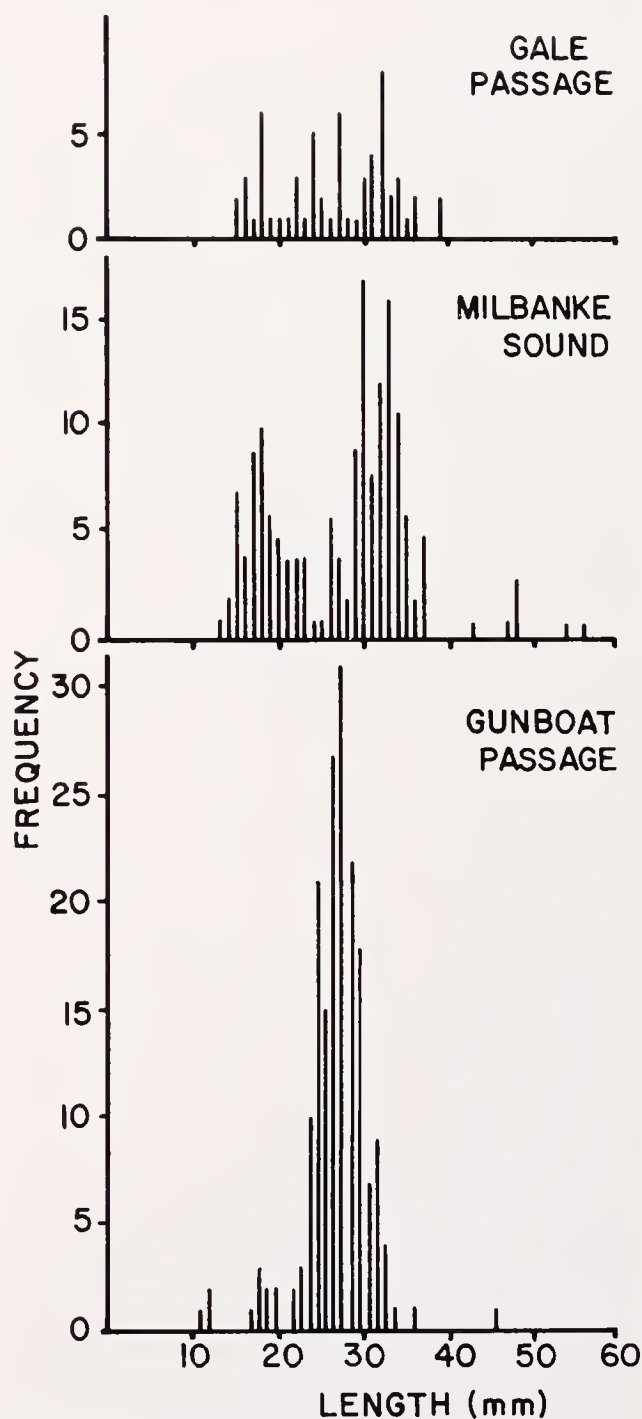


Figure 3. Length frequency distribution of Manila clams in the central coastal area of British Columbia, 1980.

Manila clam populations at Bella Bella in the central coastal area may have originated from more than one source. Adult clams may have been planted by unknown individuals, but this seems unlikely because the clams occur over a wide area suggesting a more general introduction. Transplants of Pacific seed oysters were made in 1967 to several locations along the British Columbia coast in raft culture experiments (Quayle 1971). One location was Pruth Bay, Calvert Island, about 25 km south of Spider Anchorage. It is possible that Manila clams were transported to Pruth Bay along with the seed oysters and spawnings from these clams produced larvae which drifted northward up the coast. However, surveys in the Pruth Bay area in 1970 failed to find any clams and surface currents do not favor this route of dispersal. Clam populations at Bella Bella may have originated in the Quatsino Sound area, or from spawnings at Spider Anchorage, or areas in between because water temperatures, currents, and the larval period are suitable for transporting larvae there from any of the three sources. However, unless the Spider Anchorage population is much more extensive than recorded in 1973, it is likely that the central coast population originated from the Quatsino Sound area because too few larvae would be produced from the sparse population at Spider Anchorage.

The population structure of Manila clams in the Bella Bella area (Figure 3) shows it is either continually receiving (virtually yearly) introductions of larvae from southern areas or, more likely, successful local reproduction is occurring. Water temperatures at McInnes and Ivory islands (Dodimead 1980) are sufficient for reproduction, particularly in years with above-average summer water temperatures. Water temperatures in protected local areas with restricted circulation would be higher than at monitoring stations. Annual reproduction may not occur in years of low water temperatures, but the population may obtain sporadic spatfall from other areas such as Quatsino Sound. Expansion of the population may depend not only on summer water temperatures, but also on the severity of subsequent winters. Williams (1978) reported that clams which set in Puget Sound in September overwintered at under 2-mm shell length and were located in the top few centimeters of sediment. Because Manila clams inhabit a high intertidal beach position, they could suffer mortalities during harsh winters.

Introduction of Manila clams into the Queen Charlotte Strait-Alert Bay area is less well explained. Adult clams may have been taken from the Strait of Georgia to the Alert Bay area via seed oyster transplants or by recreational clammers. However, this seems unlikely because Manila clams occur over a wide area which indicates a more general introduction.

Another route would be north through Seymour Narrows-Yuculta Rapids region, but this also seems unlikely. If Manila clams had spread to the Alert Bay area via this route, one would have expected it to have occurred in the late 1940's or early 1950's (particularly during a warm summer such as 1958), rather than in the mid-1960's.

Manila clams occur at Port Harvey but not in Port Neville (Bourne, unpublished) which is about 17 km to the south of Port Harvey (i.e., towards Seymour Narrows) in Johnstone Strait. If dispersal had been north through the Seymour Narrows-Yuculta Rapids area, one would expect the clams to populate beaches in the Port Neville area before those in the Port Harvey area.

The most plausible route for the introduction into the Alert Bay area would be from spawnings at Quatsino Sound. Current patterns (Thompson and Van Cleve 1936, Dodimead and Hollister 1962, Dodimead 1980) show larvae could be transported to the mainland side of Queen Charlotte Strait and then into the Alert Bay area. To date, Manila clams have been found only near the mainland side of Queen Charlotte Strait and not on the Vancouver Island side, which supports this hypothesis.

The size structure of this population indicates that little if any local reproduction has occurred. Water temperatures (Hollister and Sandes 1972) are too low to permit widespread reproduction, but local reproduction may occur in embayments such as Port Harvey or Booker Lagoon where water circulation is restricted and water temperatures higher. Larvae from such local spawnings could populate other beaches in the Alert Bay area but surveys show that has not been extensive.

#### REPRODUCTIVE CYCLE

Ohba (1959) reported that Manila clams spawned twice a year in different parts of Japan, usually late spring and again in early-to-late fall. Yamamoto and Iwata (1956) found a single spawning period which lasted from July to September in Hokkaido. Holland and Chew (1974) reported an extended spawning period for clams in Puget Sound; spawning began in late June and continued at intermittent periods during the summer and into autumn. Williams (1978) confirmed the extended spawning season in Puget Sound and observed two periods of settlement, a minor one in July and a major one in September.

The reproductive cycle of Manila clams in the Strait of Georgia, as determined by examination of adults and analysis of larval development in the plankton, is similar to that in Puget Sound. Adults are ripe in early June and some spawning occurs in mid-to-late June because umbo larvae have been found in the plankton at the end of June; larvae have been found in the plankton until September. On the western coast of Vancouver Island, the reproductive cycle is probably similar to that in the Strait of Georgia, although spawning may be somewhat later because water temperatures are lower, particularly as one proceeds northward.

Gonad samples were taken from the largest Manila clams from three beaches in the central coastal area in late May and early June 1980 to determine stages of gonadal development. Gonads were preserved in Davidson's solution, blocked in paraffin, sectioned at 5  $\mu$ m, stained with haematoxylin-eosin, and examined microscopically. The

five stages of the reproductive cycle described by Holland and Chew (1974) were used to classify development. The sample size was relatively small and only a single sample was taken, but the results are included here for information (Table 1).

TABLE 1.

Stages of gonadal development in Manila clams at three locations in the central coastal area of British Columbia.

Date 1980	Location	Early Active	Late Active	Ripe	Partially Spent	Spent
May 29	Leighton Island	0 M	12 M	0 M	0 M	0 M
	Milbanke Sound	0 F	4 F	0 F	5 F	4 F
May 30	Horsefall Island	0 M	10 M	0 M	0 M	0 M
	Milbanke Sound	0 F	6 F	0 F	1 F	1 F
June 3	Gunboat Passage	0 M	7 M	0 M	0 M	0 M
		0 F	3 F	4 F	1 F	0 F

No clams were in the early active stage. All males and most females were in the late active stage. Five females were spent; they probably spawned the previous summer and had not begun to regenerate gonadal tissue in 1980. Seven females were partially spent; again, these clams probably did not completely spawn out during the previous summer and had not begun to regenerate gonadal tissue in 1980. Four females from Gunboat Passage were ripe. It is unlikely these clams developed to the ripe stage in 1980, rather they were probably ripe in 1979, did not spawn, and remained in a ripe condition throughout the winter and spring. This condition has been observed frequently in the butter clam *Saxidomus giganteus* (Deshayes) in British Columbia.

Monthly mean surface water temperatures at McInnes and Ivory islands (Hollister and Sandes 1972, Dodimead 1980) are about 10°C in May. Holland and Chew (1974) observed that Manila clams in Puget Sound were in the late active stage in mid-April when water temperatures were about 8°C, hence development to the late active stage at the end of May at Bella Bella is not unexpected. Spawning would probably not occur until August in years with normal or above-normal temperatures when the time-temperature period for gametogenesis is sufficient and water temperatures high enough for spawning. In below-normal summers, complete gametogenesis and spawning probably do not occur.

#### GROWTH

Growth was measured for Manila clams from various locations in the Strait of Georgia, the western coast of Vancouver Island, Alert Bay, and central coastal areas. Growth was determined by measuring shell length at winter annuli (straight-line distance between the anterior and posterior margins of annuli) with calipers to the nearest mm (Quayle and Bourne 1972). Most clams from British

Columbia have distinct winter annuli. The sample size from Alert Bay was limited but is included here for comparison. Von Bertalanffy (1938) growth parameters were calculated (Table 2), and the resultant curves drawn through the points on Figure 4.

Growth of Manila clams, as shown by  $k$ , was fastest in the Strait of Georgia, followed by the western coast of Vancouver Island; growth was slower in the central coastal area and slowest in the Alert Bay area. Calculating growth using the method of Gallucci and Quinn (1979) shows a higher omega value for clams from the Strait of Georgia, followed in order by those from the western coast of Vancouver Island, the central coastal area, and the Alert Bay area. Manila clams attain legal commercial shell length of 38 mm in about 3.5 years in the Strait of Georgia; in about 4 years on the western coast of Vancouver Island; in about 5 years in the central coastal areas; and in 5.5 years in the Alert Bay area. Growth is fastest where water temperatures are highest (i.e., the Strait of Georgia), and slowest where water temperatures are lowest (i.e., the Alert Bay area).

The  $L_{\infty}$  of the Von Bertalanffy equation shows that although Manila clams in the central coastal and Alert Bay areas have slower growth rates than those in the Strait of Georgia, they can attain a larger size which is similar to what Weymouth and McMillan (1930) observed in razor clam populations along the western coast of North America.

#### FUTURE DISPERSAL

No physical barriers exist to deter future expansion of Manila clam populations in British Columbia; dispersal will depend on environmental parameters. Low water temperatures, slow growth rates, and cold winter temperatures will probably deter development of extensive Manila clam populations in the Queen Charlotte Strait-Alert Bay area. In the central coastal area, reproduction has apparently occurred. Surface water currents there drift northward along the eastern side of Hecate Strait (Dodimead and Hollister 1962) and could carry larvae northward. This was the dispersal route of another exotic, *Mya arenaria* Linnaeus, which spread northward into Alaska and across Dixon Entrance into the Queen Charlotte Islands (Quayle 1964). However, monthly mean surface water temperatures at Bonilla and Triple islands rarely attain 14°C which may be too low to permit gametogenesis, spawning, and larval development of Manila clams. The high intertidal beach position during harsh winters could also cause extensive mortalities of any spat that might settle there. Development of an extensive clam population north of the central coastal area of British Columbia will probably be slow.

The possibility exists that a race of Manila clams has developed in these northern areas which undergoes gametogenesis and spawns at colder water temperatures than reported by Mann (1979). If this has happened, an extensive clam population could develop more quickly in this and the Alert Bay areas.

TABLE 2.

Parameters of the von Bertalanffy growth curve,  $L_{\infty} = L_{\infty} [1 - B \exp(-Kt)]$ , as obtained from measurements of shell length at winter annuli of Manila clams from various locations in British Columbia.

Location	k	$\pm t \times SE$	L	$\pm t \times SE$	$t_0$	$\pm t \times SE$	$\omega$
<b>STRAIT OF GEORGIA</b>							
Von Donop Inlet	0.22	0.061	67.7	9.4	-0.02	0.191	14.89
Savary Island	0.30	0.053	55.1	3.4	+0.09	0.272	15.98
Kulleet Bay	0.39	0.025	47.6	1.0	+0.02	0.051	18.56
<b>WEST COAST OF VANCOUVER ISLAND</b>							
Atleo River	0.23	0.072	62.5	8.3	+0.01	0.304	14.38
Hesquiat Harbor	0.29	0.172	56.1	13.8	+0.002	0.445	15.71
Toquart Bay	0.31	0.104	49.1	7.8	-0.14	0.187	15.22
Hilliers Island	0.26		60.6		-0.18		15.76
<b>CENTRAL COAST</b>							
Horsefall Island	0.15	0.116	75.1	34.5	+0.02	0.416	11.25
Gunboat Passage	0.14	0.392	84.5	159.3	+0.04	0.111	11.83
Lady Trutch Island	0.17	0.075	69.5	15.5	-0.04	0.389	11.82
Bella Bella combined	0.15	0.044	73.0	11.1	-0.02	0.239	10.95
<b>ALERT BAY</b>							
Alert Bay combined	0.14	0.062	68.8	14.3	-0.28	0.522	9.63

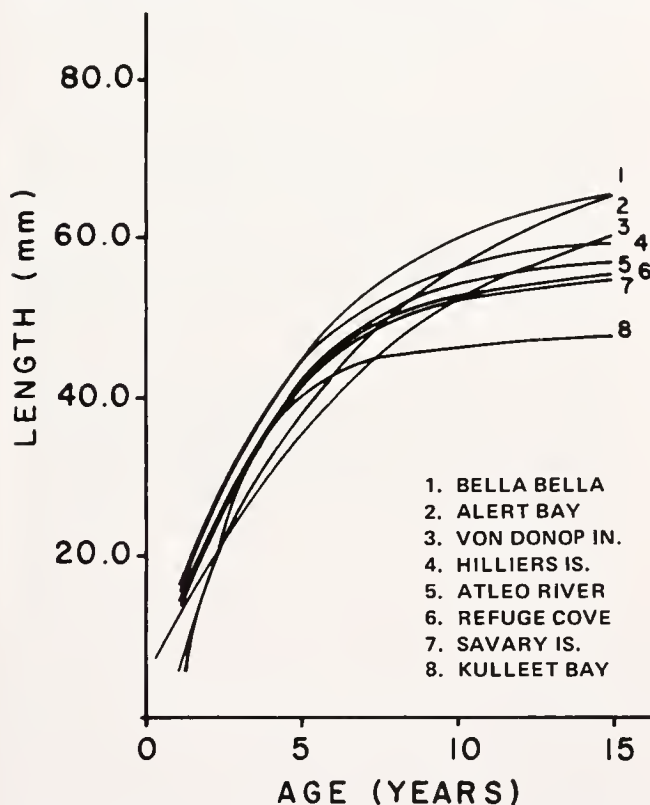


Figure 4. Predicted length at age Von Bertalanffy curves for Manila clams from several locations in British Columbia.

#### SUMMARY

Manila clams were first found in British Columbia at Ladysmith Harbour in 1936 and, within 30 years, had

spread throughout the Strait of Georgia and along the western coast of Vancouver Island where they are now abundant.

Transplants of Manila clams to two northern areas of the Province were not successful.

In the late 1960's and early 1970's, Manila clams spread to the Queen Charlotte-Alert Bay and central coastal areas. A reproducing population of clams is now established in the central coastal area.

The reproductive cycle of Manila clams in the Strait of Georgia is similar to that in Puget Sound; clams spawn from mid-to-late June until September. A single sample from the central coastal area shows that reproduction can occur, particularly in warmer years, and spawning probably occurs in August.

Growth of Manila clams is fastest in the Strait of Georgia and slowest in the Alert Bay area.

Future dispersal northward is possible but will depend on environmental parameters such as water temperatures, currents, and severity of winters after spatfall. Development of a race of Manila clams that spawn at lower temperatures would hasten dispersal northward.

#### ACKNOWLEDGMENTS

Sincere appreciation is expressed to Dr. D. B. Quayle for determinations of gametogenesis, use of unpublished material, and review of the manuscript. I am also indebted to Mr. A. J. Dodimead for frequent consultations on oceanographic details and use of unpublished data. Ms. S. Farlinger assisted with the field work and in the analysis of the growth data.

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## THE SPAWNING SEASON OF FOUR SPECIES OF CLAMS IN OREGON<sup>1</sup>

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**ABSTRACT** Annual reproductive cycles are described for the gaper clam *Tresus capax* (Gould), the butter clam *Saxidomus gigantea* (Deshayes), the littleneck clam *Venerupis staminea* (Conrad), and the cockle clam *Clinocardium nuttallii* (Conrad). Clams used in this study were collected from Yaquina and Tillamook bays on the Oregon coast. Histological preparations made during the 2-year study indicate that the primary spawning season for gaper clams is from late January through April, for butter clams from February to July, for littleneck clams from March through August, and for cockle clams from June to October.

### INTRODUCTION

Along the Oregon coast clams are extensively harvested by recreational clambers during periods of low tide. This activity is limiting the availability of clams in intertidal areas, while subtidal clam populations are still plentiful, as clams are now being taken commercially in Coos and Yaquina bays. It is possible that supplemental seeding will be necessary to maintain the recreational fishery. Detailed knowledge of the spawning seasons of the gaper clam *Tresus capax* (Gould), the butter clam *Saxidomus gigantea* (Deshayes), the littleneck clam *Venerupis staminea* (Conrad), and the cockle clam *Clinocardium nuttallii* (Conrad), is lacking for the Oregon coast. The purpose of this study was to provide information on timing and duration of the reproductive activity in these clams. Gametogenic development was followed monthly through histological sections of gonadal tissue.

### MATERIALS AND METHODS

This study began in February 1979, and was completed in January 1981. Clams were collected from Yaquina Bay at Newport on the central Oregon coast, and from Tillamook Bay about 137 km (85 mi) north of Newport, OR. During low tide, clams were dug by hand or raked; at other times they were collected by scuba diving or from the commercial harvest.

Clams were brought to the Oregon State University (OSU) Marine Science Center where samples of gonads, approximately 1 cm<sup>3</sup> in size, were taken from the same part of the gonad each time and prepared for histological study. The tissue was fixed in Bouin's solution for 24 to 48 hours, dehydrated in alcohol, cleared in toluene, embedded in paraffin, sectioned at 6  $\mu$ m, and then stained with Mayer's hematoxylin and eosin.

Breed-Willeke and Hancock (1980) studied the growth and reproduction of the gaper clam *T. capax* in Yaquina Bay. Samples were collected from April 1975 through February 1977, at four locations: three subtidal and one intertidal. The same histological techniques were used in that study. Because the study did not find statistically significant differences between the samples from different locations, the data were compiled to give a monthly sample size of 80 or more. The minimum monthly sample size for the other clam species was 10.

The sex of a clam in spawning condition can easily be determined by piercing the gonad and observing the granular material containing ova from females or the milky material containing sperm from males. The sex of each clam was identified and the maturity of the gametes determined. The gonadal development stage was determined by counting oocytes and ova in histological sections. Oocytes and ova were counted in the follicles until at least 100 cells were included. The stages of maturation were: (1) Small oocytes attached to the follicle wall and staining dark purple. (2) Growing oocytes moving from the wall of the follicle toward the lumen. (These are club shaped with the narrow end still attached on the follicle wall, and stain lighter than small oocytes.) (3) Fully developed ova staining pink and filling the lumen. When clams appeared mature, spawning was attempted using *Pseudosochrysis paradoxa* Dupuy as a stimulant (Breese and Robinson 1981) to determine whether clams were ripe.

### RESULTS

The proportions of ova in the gonads of *T. capax*, *S. gigantea*, *V. staminea*, and *C. nuttallii* are presented graphically in Figures 1, 2, 3, and 4, respectively. Based upon these data, the most probable spawning periods for the several species are: *T. capax*, January through March; *S. gigantea*, March through June; *V. staminea*, April through August; and *C. nuttallii*, June to October.

### DISCUSSION

Clark et al. (1975) studied *T. nuttallii* (Conrad) in Elkhorn Slough, CA, and stated that it is a winter spawner.

<sup>1</sup> This work was supported by the National Oceanic and Atmospheric Administration, Office of Sea Grant, through Oregon State University Sea Grant Program under grant No. NA79AA-D-00106. Technical Paper No. 5940, Oregon Agriculture Experiment Station.

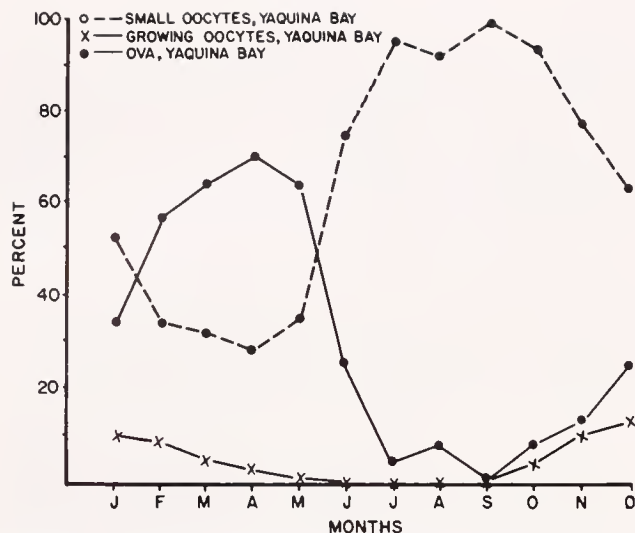


Figure 1. Annual reproductive cycle of the gaper clam *Tresus capax* in Yaquina Bay, Oregon.

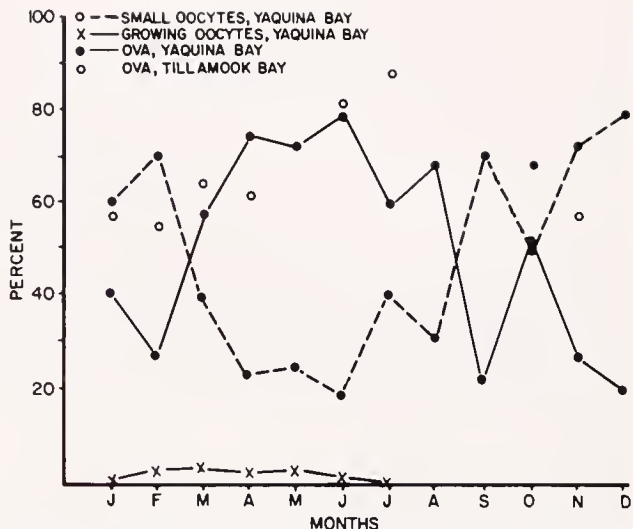


Figure 3. Annual reproductive cycle of the littleneck clam *Venerupis staminea* in Yaquina Bay and Tillamook Bay, Oregon.

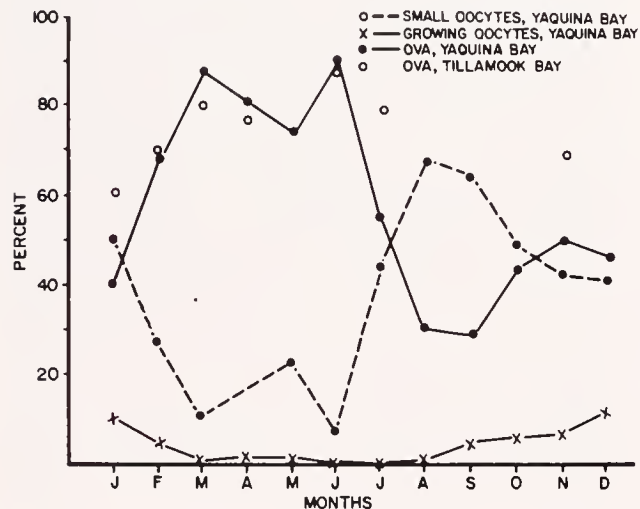


Figure 2. Annual reproductive cycle of the butter clam *Saxidomus gigantea* in Yaquina Bay and Tillamook Bay, Oregon.

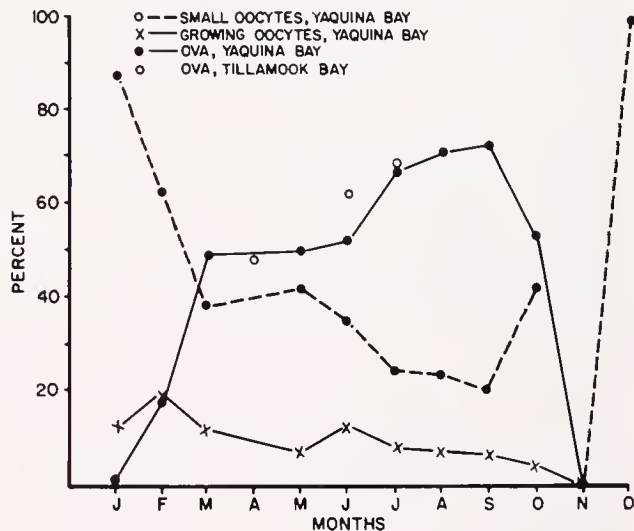


Figure 4. Annual reproductive cycle of the cockle clam *Clinocardium nuttallii* in Yaquina Bay and Tillamook Bay, Oregon.

Machell and DeMartini (1971) studied *T. capax* in Humboldt Bay, CA, and found it to be a winter spawner. They stated that spawning corresponded with minimum, seasonal temperature and salinity measurements. The temperature and salinity of Yaquina Bay water are constantly recorded at the OSU Marine Science Center and, according to these records, the annual changes appear to parallel those in Humboldt Bay.

Bourne and Smith (1972) reported that *T. capax* was a late winter and early spring spawner in the Strait of Georgia, British Columbia. This agrees with the studies in Humboldt Bay, CA, and Yaquina Bay, OR. The spawning season of this clam appears to begin somewhat later in the year in Alaska than in California.

According to Nickerson (1977), the spawning season

of the butter clam in Prince William Sound, AK, starts 3 months later than in Yaquina Bay. The spawning season of butter clams in Tillamook Bay appears to follow the one in Yaquina Bay.

The spawning season of the littleneck clam appears to last somewhat longer in the fall in Tillamook Bay than in Yaquina Bay. In November, 60% of the ova were present in Tillamook samples when the percent of ova had dropped to 25 in Yaquina Bay samples.

Samples of cockle clams were collected only a few times during the year in Tillamook Bay for comparison of gonadal development in Tillamook and Yaquina bays. The percentage of ova in Tillamook Bay cockles (Figure 4) appeared to follow very closely that of cockles from Yaquina Bay; the only difference was that spawning appeared

to be completed in Tillamook Bay about a month earlier than in Yaquina Bay.

In Yaquina Bay each of these clam species appears to have one primary spawning. Those seasons vary in length and time of year.

#### ACKNOWLEDGMENTS

We thank the following personnel from the Oregon Department of Fish and Wildlife for their help in obtaining the clam specimens: Darrel Demory, Thomas Gaumer, Gregory Robart, and Dennis Wise.

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## INCREASE IN A SURF CLAM POPULATION AFTER HYPOXIC WATER CONDITIONS OFF LITTLE EGG INLET, NEW JERSEY

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**ABSTRACT** From July through September 1976, intermittent hypoxic water conditions occurred off Little Egg Inlet, New Jersey. The following year, the estimated number of surf clams in the 100-km<sup>2</sup> area had increased seven fold, due to the increased number of 1+ year-old clams. The high survival of the 1976 cohort indicated that the population had the ability to make a rapid recovery after intermittent hypoxia. Apparently, a reduction of the number of predatory echinoderms and crustaceans during the period when the 1976 cohort set, contributed to its success.

### INTRODUCTION

The Atlantic surf clam *Spisula solidissima* (Dillwyn) has comprised over half the yearly totals of United States clam landings from the 1940's through 1977 (Ropes 1979). It ranges from the Gulf of St. Lawrence, Canada, to Cape Hatteras, NC. Ropes (1979) found that the highest concentration of clams in the New York Bight occurred off southern New Jersey in depths < 18 m.

Mass mortalities of benthic organisms occurred off the New Jersey coast in the summer of 1976 (Swanson and Sinderman 1979). Hypoxic water conditions (< 2 ppm dissolved oxygen content in water below the thermocline) developed in a 6,750-km<sup>2</sup> area, and destroyed 62% of the New Jersey surf clam resource (Ropes 1980). Garlo et al. (1979) reported that hypoxia occurred intermittently off Little Egg Inlet, NJ, from mid-July through late September 1976, and destroyed about 7% of the surf clam population.

Population estimations were made for the total number and biomass of surf clams in a 100-km<sup>2</sup> nearshore area off Little Egg Inlet immediately after hypoxia occurred and one year later. The objective was to determine the change in population size one year after the occurrence of hypoxic conditions to demonstrate the ability of a surf clam population to recover after the effects of relatively mild hypoxic conditions. Historical data including salinity, oxygen, temperature, and abundance of crustaceans and echinoderms were compared to data collected during the hypoxic period.

### MATERIALS AND METHODS

A hydraulic sampling dredge was used to collect surf clams and other macroinvertebrates in a 100-km<sup>2</sup> area off Little Egg Inlet, NJ (Figure 1). The 30-cm-wide dredge blade dug to a depth of 12.7 cm. The bag was of 51-cm iron rings and had a net liner with a 3.8-cm stretch mesh size. An 18-hp, 380-ℓ/min, high-pressure pump was used to jet water through a manifold and into the bottom sediment.

Hauls of 7-minute duration were made at each station. A Motorola Mini-Ranger System was used for navigation purposes. Mini-Ranger coordinates were recorded when the

dredge touched bottom and when it left bottom to determine the station location and distance towed. The system operated at ranges up to 37 km and the accuracy was ± 3 m (EG&G, Environmental Consultants 1974).

Organisms caught in the dredge were identified, counted, and weighed in the shell. All surf clams were divided into three categories in the field based on length and number of external annual growth marks. Small clams were < 30 mm and had no annual growth marks. Medium-size clams ranged from 30 to 79 mm and had one annual growth mark. Large clams were > 80 mm and had more than one annual growth mark. The anterior-to-posterior length of all medium and large clams up to 150 clams per collection was recorded at every station, and the length of all small clams was recorded.

Stratified random sampling surveys were conducted in September 1976 and 1977. The 100-km<sup>2</sup> study area was divided into three strata based on relative clam abundance obtained from a systematic survey (50 evenly spaced stations) made in 1975. In 1976, 50 stations were distributed among the three strata with proportionally more stations allotted to the stratum with the highest density and fewest stations allotted to the stratum with lowest density according to a procedure described by Mackett (1973). The same stations were resampled in 1977.

To locate a station, a grid was superimposed on a chart of the stratified study area and stations were selected randomly from each stratum using a random number table. Mini-Ranger coordinates were determined for each station from the chart and used in the field to locate a station.

Estimators and 95% confidence limits for the total number and weight and mean density and biomass of the surf clam population within the study area were developed with the formulae presented in Schaeffer et al. (1979).

Differences in clam density and biomass between years and between strata were tested by a two-way analysis of variance (randomized complete block design). Data were transformed using the natural logarithm of  $n + 1$ . Calculations were done with an Amdahl 480 computer using the

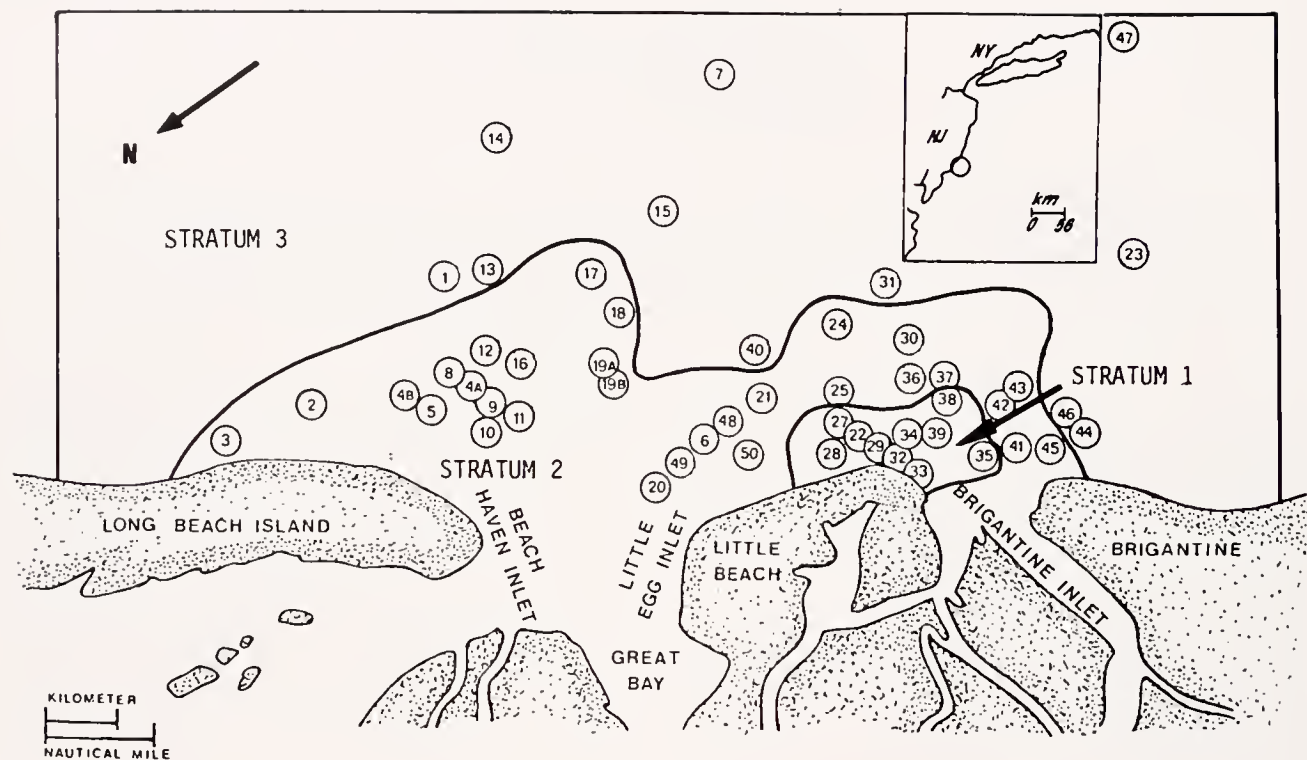


Figure 1. Stations sampled with a hydraulic clam dredge off Little Egg Inlet, NJ, in 1976 and 1977.

Statistical Analysis Systems package (Statistical Analysis Systems Institute 1979).

A  $2 \times 3$  Chi-square contingency table was used to test for differences in size-class distributions (small, medium, and large) between the two years.

Historical data collected within the study area from 1972 to 1975 were examined and compared to data collected during the hypoxic period in 1976 (Milstein et al. 1977). Epibenthic macrofauna such as the starfish *Asterias forbesi* (Desor), the rock crab *Cancer irroratus* Say, and the lady crab *Ovalipes ocellatus* (Herbst) were sampled from 1972 through 1976 with a 7.6-m semiballoon trawl with a 3.5-cm mesh body and a 1.3-cm stretch mesh liner for the cod end. A transect of three stations which ranged from 1.8 to 7.5 km southeast of Little Egg Inlet was sampled twice each month. Pooled monthly abundance (number/collection) of predatory species collected from 1972 to 1975 was compared to the monthly abundance taken in 1976 for the months in which hypoxia occurred. Mean bimonthly bottom temperature, salinity, and oxygen values were calculated from measurements made approximately 4.3 km southeast of Little Egg Inlet from 1972 to 1974, and were compared to similar data collected in 1976.

## RESULTS

In 1976, 50 samples taken from 8 to 29 September yielded 8,264 surf clams that weighed 1,555 kg. The estimated total number of clams and the 95% confidence interval were  $111.5 \times 10^6 \pm 41.2 \times 10^6$  clams for the 100-km<sup>2</sup> study area (Table 1). The estimated biomass was  $13,000 \pm 6,500$  metric tons (t).

In 1977, 50 samples taken from 12 September to 12 October yielded 28,291 clams that weighed 1,805 kg. The estimated total number of clams and the 95% confidence interval were  $793.0 \times 10^6 \pm 677.4 \times 10^6$  clams for the study area. The estimated biomass was  $18,000 \pm 32,100$  t (Table 1). A 7-fold (700%) increase in the number of clams occurred during the two years, and the biomass increased by a factor of 1.4 (140%).

Analysis of variance showed the difference between years was highly significant ( $P = 0.001$ ) for number, but not significant for biomass. Differences between strata were highly significant for both density and biomass ( $P = 0.001$ ), which indicated that stratification was helpful for determining the population estimates. No interaction between year and stratum occurred.

TABLE 1.

Population estimates of the total number and weight of surf clams in a 111-km<sup>2</sup> area off Little Egg Inlet, NJ, in late summer, 1976 and 1977.

Year	Stratum	Stratum Area (m <sup>2</sup> × 10 <sup>4</sup> )	No. of Samples	Density (No./m <sup>2</sup> )	Estimated Number		Biomass (g/m <sup>2</sup> )	Estimated Weight	
					No. × 10 <sup>6</sup>	CI* × 10 <sup>6</sup>		kg × 10 <sup>6</sup>	CI × 10 <sup>6</sup>
1976	1	178	10	3.33	5.9	3.3	545	1.0	0.6
	2	2,571	29	2.12	54.5	21.4	442	11.4	4.7
	3	8,325	11	0.61	51.1	35.1	78	0.6	4.5
Total		11,074	50	1.01	111.5	41.2	170	13.0	6.5
1977	1	178	10	13.03	22.3	13.8	663	1.2	0.7
	2	2,571	29	5.68	146.0	91.6	547	14.1	6.0
	3	8,325	11	7.49	623.8	671.0	325	2.7	31.5
Total		11,074	50	7.16	793.0	677.4	358	18.0	32.1

\*CI = 95% confidence interval.

In 1976, the mean density for the entire study area was 1 clam/m<sup>2</sup> and the biomass was 170 g/m<sup>2</sup>. In 1977, the mean density was 7 clams/m<sup>2</sup> and the biomass was 382 g/m<sup>2</sup>. The density at a station ranged from 0 to 45 clams/m<sup>2</sup> and biomass ranged from 0 to 2,260 g/m<sup>2</sup>.

The Chi-square test showed a highly significant difference ( $P = 0.001$ ) between numbers of clams comprising small, medium, and large size classes in 1976 and 1977. The abundance of medium size clams in the second year accounted for most of the difference between the two years. Medium-size clams were 60% of the total in the second year, and only 1% in the first year (Figure 2).

The six macroinvertebrate species which comprised 95% of the trawl catch from 1972 to 1975 were: *Crangon septemspinosus* (Say) (73%, 346 specimens/collection); *Echinarachnius parma* (Lamerck) (8%, 38 specimens/

collection); *Loligo pealei* Lesueur (6%, 23 specimens/collection); *Asterias forbesi* (Desor) (3%, 14 specimens/collection); *Cancer irroratus* Say (3%, 14 specimens/collection); and *Ovalipes ocellatus* (Herbst) (2%, 7 specimens/collection). Ropes (1980) considered crabs and starfish as potentially important predators of surf clams. Comparison of the pooled relative abundance (number/collection) of two crab species and starfish from 1972 to 1975 to their abundance in 1976 indicated that their numbers were substantially lower in 1976 during the hypoxic period (Figure 3). Rock and lady crabs, and starfish averaged more than 10 specimens/collection in August, September, and October 1972–1975. In August and September 1976, there was nearly an order of magnitude decrease, and they averaged less than 0.5 specimen/collection. No rock and lady crabs, or starfish were collected in October 1976.

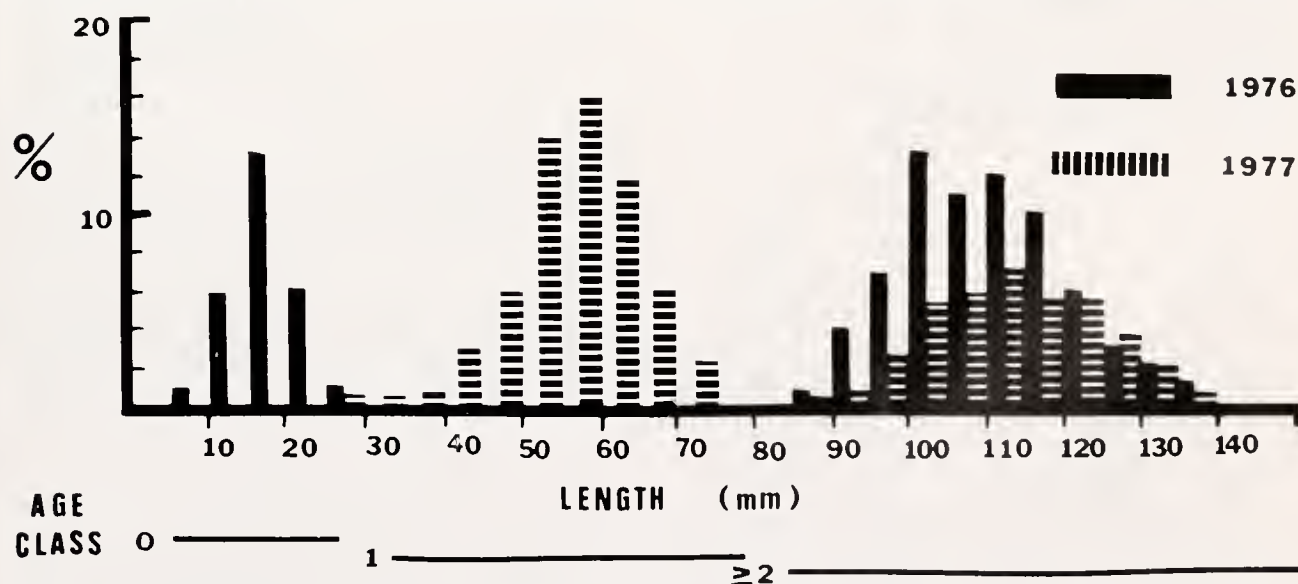


Figure 2. Anterior-to-posterior length frequency distribution of surf clams collected off Little Egg Inlet, NJ, during late summer, 1976 and 1977, with the range of size for three age classes.

Mean bimonthly bottom temperature, salinity, and oxygen values were calculated from measurements made approximately 4.3 km southeast of Little Egg Inlet in 1976 and from 1972 to 1974 (Figure 4). The most pronounced difference between 1976 and the previous years

was for the dissolved oxygen values for July and early August 1976, which were below the minimum recorded during previous years. During that period, values ranged from 0.4 to 7.1 ppm. Mean annual bottom temperature in 1976 was 11.0°C and was 11.6°C for 1972 to 1974. Except for a rapid cooling trend in the fall, the mean bimonthly temperature fell within the range of the 1972–1974 values. Average bottom salinity was 31.0 ppt in 1976 and 29.9 ppt in 1972–1974. Salinity in 1976 was relatively high from May through mid-October.

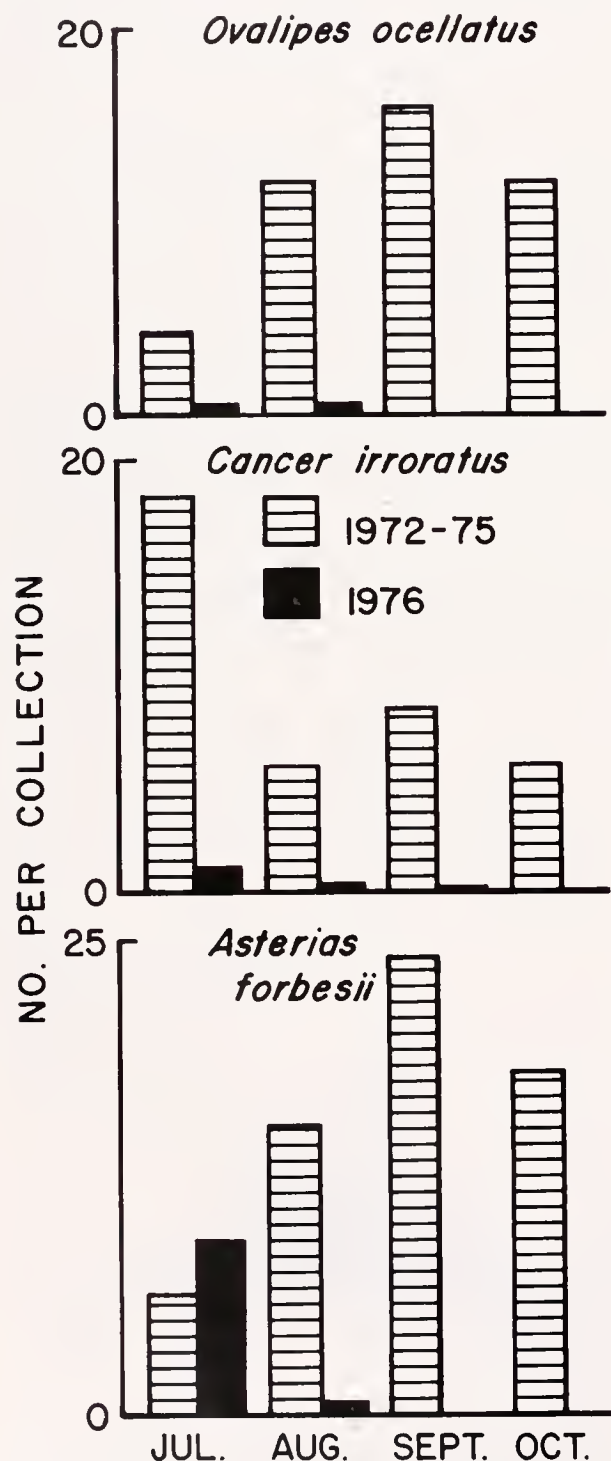


Figure 3. Relative abundance of common invertebrates collected by trawl off Little Egg Inlet, NJ, for pooled data from 1972 to 1975 versus 1976.

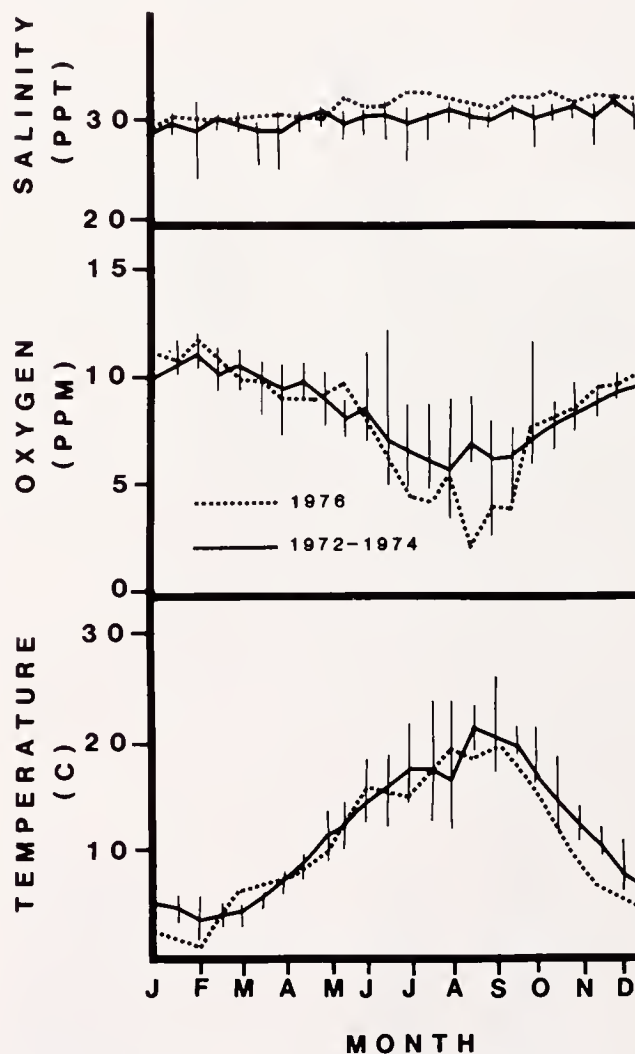


Figure 4. Mean bimonthly bottom temperature, dissolved oxygen, and salinity values taken 4.3 km off Little Egg Inlet, NJ, in 1976 (---), and mean range of values for 1972–1974 (—). Taken from Ichthyological Assoc., Inc. (unpublished).

#### DISCUSSION

One year after hypoxic conditions killed 7% of the surf clams in the study area (Garlo et al. 1979), the surf clam population density increased by 700%. The increase was due to the presence of more medium-size (30 to 79 mm)

clams in the second year. Jones et al. (1978) presented growth curves for inshore (1.8 km from shore) and offshore (17.5 km) New Jersey surf clams. They found the mean length of 1+ year-old inshore and offshore clams to be 55 and 62 mm, respectively. The mean length of medium-size clams taken off Little Egg Inlet was 56 mm and the clams had one external annual growth mark. They represented the survivors of the 1976 cohort.

Over 300 dredge samples were taken off Little Egg Inlet from 1972 to 1975. Medium-size clams (1+ years old) comprised 100% of the clams collected in 1972 and 60% in 1977, but were very scarce in other years (Garlo and Hondo 1973; Garlo et al. 1974, 1975, 1976). Haskin et al. (1979b) summarized data from inshore dredge surveys taken annually off southern New Jersey from 1972 to 1978. They found 1+ year-old clams were only common in 1972 and 1977, and used their abundance as evidence of significant survival of clams which had settled the previous year. Franz (1976) and Jones (1981) found irregular and infrequent survival of a cohort based on age-frequency distributions of dredged clams.

From 1972 to 1975, small, recently settled surf clams (1–29 mm) were abundant in grab samples taken monthly off Little Egg Inlet from May to July, but by August were nearly absent (Garlo and Hondo 1973; Garlo et al. 1974, 1975, 1976). Haskin et al. (1979b) found that recently settled clams collected in grab samples taken within 6 km of shore off southern New Jersey from 1974 to 1976 were most abundant from May through July (mean density was 200 to 400 clams/m<sup>2</sup>), but very few were collected by late summer and fall. Haskin et al. (1979a) summarized grab data collected from nearshore New Jersey waters and concluded that general setting and early growth of juveniles occurred every year.

Muus (1966, 1973) believed predation was the cause of the disappearance of a dense (ca 8,500/m<sup>2</sup>) set of the European surf clam *Spisula subtruncata* (DaCosta). The set occurred from June to August and rapidly disappeared within two months. The starfish *Astropecten irregularis* (Pennant) feeds on European *Spisula* spp., and one individual may destroy 30,000 spat of *S. subtruncata* annually (Cristensen 1970). Thorson (1966) observed that the heart urchin *Echinocardium cordatum* (Pennant) ingested *S. subtruncata* of about 1 mm in length and may consume 224 young in the 4-week period when small sizes are available. The hermit crab *Pagurus bernhardus* (Linnaeus) also preyed upon *Spisula* spat. Ropes (1980) suggested that crabs were also potential predators of surf clams, and Tagatz (1968) found three mactrid species in the stomachs of the blue crab *Callinectes sapidus* Rathbun.

Garlo et al. (1979) reported the immediate impact of hypoxia on organisms collected off Little Egg Inlet with a grab, dredge, and trawl, and found echinoderms and crustaceans had higher mortalities than bivalves and gastropods. Boesch et al. (1977) and Reid and Radosh (1979) likewise concluded that echinoderms and crustaceans were severely affected by hypoxic conditions, but that bivalves and polychaetes were resistant based on the percentages of recently killed organisms taken in grab samples. For example, Garlo et al. (1979) found 88% of the sand dollars, 47% of the starfish, and 8% of the crustaceans in grab samples were recently dead, but only 1% of the surf clams and less than 1% of the polychaetes were recently dead. Likewise, the numbers of crabs and starfish collected by trawl in the study area during the hypoxic period were very depressed when compared to the numbers taken from 1972 to 1975. The most common potential predators were starfish, and rock and lady crabs, and their abundance during the hypoxic period was at least an order of magnitude lower than it was for pooled data from 1972 to 1975. No mortalities were observed for the Atlantic moon snail *Polinices duplicatus* (Say) or the northern moon snail *Lunatia heros* (Say) collected in the study area during the hypoxic period (Milstein et al. 1977).

The significant increase in the number of clams the year after hypoxia was probably caused by an unusual combination of biotic and abiotic factors. Hypoxia occurred intermittently from July through September 1976, at a time when recently settled surf clams (< 30 mm) were most abundant. Starfish and crustaceans which were unable to move away from hypoxic water incurred higher mortality than surf clams, and by the end of the hypoxic period none of the common predators were collected by trawl. It appears that the abundance of echinoderm and crustacean predators was reduced by hypoxia, which contributed to the relatively high survival of the 1976 cohort.

#### ACKNOWLEDGMENTS

I express my sincere appreciation to Dr. M. T. Huish, of the North Carolina Cooperative Fisheries Research Unit at North Carolina State University, for his guidance and encouragement during analysis of these data. Dr. W. H. Swallow and Mrs. F. L. Childers, of the Department of Statistics at North Carolina State University, gave valuable advice on analytical procedures and computer programming, respectively. Data were collected by myself and other members of the staff of Ichthyological Assoc., Inc. Data collection was supported by Public Service Electric and Gas Company of New Jersey.

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## PRELIMINARY OBSERVATIONS ON THE USEFULNESS OF HINGE STRUCTURES FOR IDENTIFICATION OF BIVALVE LARVAE

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**ABSTRACT** Difficulties associated with discrimination of bivalve larvae isolated from plankton samples have long hampered both applied and basic research efforts in estuarine and open coastal marine environments. The vast majority of practical barriers to identification of larval bivalves may be eliminated through routine optical microscopic examination of the hinge apparatus of disarticulated larval shells. Representative micrographs of various ontogenetic stages of larval hinge development are presented for 12 genera (*Mytilus*, *Geukensia*, *Crassostrea*, *Placopecten*, *Argopecten*, *Mya*, *Spisula*, *Mulinia*, *Ensis*, *Arca*, *Arctica*, and *Mercenaria*) from 9 bivalve superfamilies (Mytilacea, Ostreacea, Pectinacea, Myacea, Mactracea, Solenacea, Arcacea, Arcticacea, and Veneracea). The larval hinge apparatus (provinculum), by itself, is generally useful for superfamilial separation. When coupled with a consideration of gross shell shape, detailed examination of hinge line structures often permits generic, or even specific, identification. A format is suggested for organization of qualitative morphological life history data that will provide an adequate basis for comparison of the larval stages of various species of bivalves.

### INTRODUCTION

An inability to identify bivalve larvae within the plankton has long hampered both applied and basic research efforts in estuarine and open coastal marine environments (Werner 1939; Jørgensen 1946; Sullivan 1948; Rees 1950; Loosanoff and Davis 1963; Loosanoff et al. 1966; Chanley and Andrews 1971; Lutz and Jablonski 1978a,b, 1979, 1981; Lutz and Hidu 1979; Jablonski and Lutz 1980; Le Pennec 1980). For example, as a result of existing practical barriers, detailed studies concerning spatfall predictions for aquacultural and fisheries management purposes have been extremely limited (for discussions, see Wisely et al. 1978, Lutz and Hidu 1979, Le Pennec 1980). Year-to-year fluctuations in larval abundance and juvenile recruitment often are not possible to define or predict because of the present inability of researchers to discriminate individual larval or early post-larval specimens with a high degree of certainty. Similarly, it has been virtually impossible in routine plankton identification studies to assess the impact of various environmental perturbations (natural "disasters," chemical pollutants, thermal discharges, oil spills, dredge spoil dumping, entrain-

ment through industrial cooling systems, etc.) on the larvae of individual species of bivalves. While a few keys for larval identification do exist (e.g., Chanley and Andrews 1971), their usefulness is limited and, at the present time, it is not possible to identify unambiguously the larvae of many bivalve species, particularly at the early (straight-hinge) developmental stages, because of the great morphological similarity of articulated shells. We offer in this paper an approach designed to eliminate many of the existing barriers to larval bivalve identification. Emphasis is placed on the usefulness of hinge (provinculum) structures in discriminating the early life-history stages of various species of bivalve molluscs.

In recent years, various workers have employed both optical and scanning electron microscopy to describe in detail the larval hinge structures of several bivalves and have suggested that such structures may be diagnostic at the generic, or even specific, level (Chanley 1965, 1969; Turner and Johnson 1969; Scheltema 1971; Pascual 1971, 1972; LaBarbera 1975; Boyle and Turner 1976; Culliney and Turner 1976; Dinamani 1976; Le Pennec and Masson 1976; Booth 1977, 1979a,b; Siddall 1977, 1978; Le Pennec

1978, 1980; Lutz and Jablonski 1978a,b, 1981; Carriker and Palmer 1979; Lutz and Hidu 1979; Chanley and Dinamani 1980; Jablonski and Lutz 1980). Despite these recent advances, much of the morphological data obtained over the past few years has not been presented in an adequate or sufficiently consistent format to permit unambiguous identification of early life-history stages. In this collaborative paper, we present representative micrographs of various ontogenetic stages of larval hinge development of nine bivalve superfamilies and suggest a format for organization of qualitative morphological life-history data that will provide an adequate basis for comparison of the planktonic stages of various species of bivalves.

## MATERIALS AND METHODS

### Culture Techniques

Sexually mature adults of the bivalves were obtained from the following locations: *Mytilus californianus* Conrad—Puget Sound, Washington; *Geukensia demissa* (Dillwyn)—Wachapreague, Virginia; *Crassostrea virginica* (Gmelin)—Cape May, New Jersey; *Placopecten magellanicus* (Gmelin)—Damariscotta River, Maine; *Argopecten irradians* (Lamarck)—Cape Cod Bay, Massachusetts; *Mya arenaria* L.—Damariscotta River, Maine; *Spisula solidissima* (Dillwyn)—Rhode Island (open coast); *Mulinia lateralis* (Say)—Cape May, New Jersey; *Ensis directus* Conrad—Damariscotta River, Maine; *Arca noae* L.—northern Adriatic Sea (Istrian Peninsula, Yugoslavian coast); *Arctica islandica* (L.)—New Jersey (open coast) and Rhode Island (open coast); and *Mercenaria mercenaria* (L.)—Damariscotta River, Maine, and Wachapreague, Virginia; and *Diplothyra smithii* Tryon—Mississippi Sound, Mississippi.

Spawning was induced using standard techniques developed by various workers (see Loosanoff and Davis 1963, Bayne 1965, Morse et al. 1977) or, in the case of *Arctica islandica*, using the ammonium hydroxide treatment described by Loosanoff and Davis (1963) and Landers (1976) (i.e., 15 to 30-minute exposure to a solution of 3 ml of 0.1N  $\text{NH}_4\text{OH}$  for every 100 ml of egg culture, followed by addition of stripped sperm).

### Scanning Electron Microscopy

Larval specimens were sampled at frequent intervals (frequency dependent upon the growth of organisms since the previous sampling period) from the various cultures of each species and placed in distilled water for 30 minutes (see Calloway and Turner 1978). Immediately following this treatment, specimens were preserved in 95% ethanol. After various lengths of time (up to 2 months), specimens were removed from the ethanol, rinsed in distilled water, and immersed in a 5% solution of sodium hypochlorite (Rees 1950) for approximately 10 minutes to facilitate separation of shell valves. After rinsing in distilled water, disarticulated valves were mounted on copper tape, coated

(under vacuum) with approximately 400 Å of gold-palladium or a combination of gold and carbon, and examined under an ETEC Autoscan scanning electron microscope. Care was taken to achieve consistent orientations of shell valves prior to photographing: each specimen was carefully manipulated under the microscope so that four points, each 90° apart, along the edge of the shell margin were in the exact same plane of focus at a magnification of approximately 9,000: when this is done, it can be calculated that the tilt of a specimen in any direction is less than 2°. This technique provides a means of obtaining a consistent, repeatable orientation, which, in turn, provides a basis for accurately comparing the gross shell morphometry of various species.

## RESULTS

Representative scanning electron micrographs of disarticulated larval shell valves at various stages of development are depicted in Figure 1. Higher magnification micrographs of the hinge region of all but one (i.e., Figure 1C') of these specimens are presented in Figure 2. These micrographs illustrate the striking differences in provinculum morphology among 12 genera (*Mytilus*, *Geukensia*, *Crassostrea*, *Placopecten*, *Argopecten*, *Mya*, *Spisula*, *Mulinia*, *Ensis*, *Arca*, *Arctica*, and *Mercenaria*) from 9 bivalve superfamilies (Mytilacea, Ostreacea, Pectinacea, Myacea, Mactracea, Solenacea, Arcacea, Arcticacea, and Veneracea). The morphology of the hinge ranges from distinctly taxodont dentition in the case of the Mytilacea, Arcacea, and Pectinacea to a lack of prominent denticular structures in the Mactracea, Veneracea, and Arcticacea. The provincular structures seen in the specimens depicted in Figures 1 and 2 are also present (although often reduced) in the early (straight-hinge) developmental stages (Figure 3).

## DISCUSSION

An extensive literature exists on the identification of bivalve larvae. For over one half of a century, workers have attempted to define larval morphological characters diagnostic at various systematic levels (for discussions, see Stafford 1912; Odhner 1914; Lebour 1938; Werner 1939; Jørgensen 1946; Sullivan 1948; Rees 1950; Miyazaki 1962; Loosanoff and Davis 1963; Newell and Newell 1963; Loosanoff et al. 1966; Chanley and Andrews 1971; Le Pennec 1978, 1980; Lutz and Jablonski 1978a,b, 1979, 1981; Lutz and Hidu 1979; Chanley and Chanley 1980). The larval characteristics generally used in routine plankton identifications are shell length, height, and depth, as well as length of the "straight-hinge line" (Loosanoff et al. 1966, Chanley and Andrews 1971, Chanley and Chanley 1980). Differences in larval shell shape, color, and texture have also been of assistance, as have the presence or absence of a byssal notch, eyespot, or apical cilia ("apical flagellum") (Chanley and Andrews 1971, Culliney et al. 1975, Turner and Boyle 1975). In the present study we have presented a number of representative micrographs depicting striking

differences in the morphologies of the larval hinge apparatus of certain bivalve species, as well as subtle differences in the shell shape of these organisms. We have attempted to present the micrographs in a manner (i.e., consistent orientation) that will provide an adequate basis for comparing the morphologies of different species. While differences among various taxa are often subtle, we believe that they can be defined, permitting unambiguous identification at the specific level. For example, the hinge structures of larval stages of *Arctica islandica* closely resemble those of corresponding stages of *Mercenaria mercenaria* (see Figures 1 and 2), as well as various other species within the family Veneridae (see Le Pennec 1978, 1980). (Interestingly, such striking similarities in early ontogenetic development suggest a closer relationship between the arctiids and venerids than has heretofore been proposed.) Despite such similarities, careful examination of the fine structures of the hinge of *A. islandica* illustrated in Figure 2G reveals subtle differences that permit discrimination of early life-history stages of this species and those of *M. mercenaria* (Figure 2H), as well as those of other venerids. It should also be emphasized here that, while we have presented scanning electron micrographs of the hinge apparatus of selected organisms, a scanning electron microscope is not necessary to observe even fine hinge structures. Such structures are readily visible under a normal, optical compound microscope equipped with a high-intensity reflected light source. Scanning electron microscopy, however, is necessary to depict photographically the three-dimensional structure of the hinge region. In routine optical microscopic studies, the disarticulated shells must be viewed in several planes of focus to discern the subtle morphological details seen in Figures 1 through 3.

We suggest that in future descriptive studies morphological data should be organized into a format that includes

not only the "minimal information" recommended by Chanley and Andrews (1971, pp. 107–109) for "detailed descriptions of laboratory-reared bivalve larvae," but also detailed scanning electron micrograph sequences of the hinge structure and gross shell morphology of the various larval stages. It is imperative that such descriptions include micrographs of all the ontogenetic stages of larval development from the Prodissoconch I through settlement and metamorphosis rather than merely representative micrographs such as those that have been included in this introductory presentation (see also, Lutz et al. 1982). The use of such a comprehensive format for presentation of life-history data should help eliminate most of the practical barriers to the identification of early stages of bivalve molluscs.

#### ACKNOWLEDGMENTS

We thank Jean Watkinson and Rosa Van Dessel for their kind assistance with larval rearing activities; John N. Kraeuter for critically reviewing the manuscript; the Department of Zoology, Rutgers University, for use of facilities; Scott M. Gallagher for many helpful suggestions; Alan S. Pooley for technical assistance with the scanning electron microscopy; and Olga Jackiw and Dorothy Cornelisse/Scott for typing the manuscript. New Jersey Agricultural Experimental Station, Publication No. D-32401-2-82, supported by State funds and by NSF grant EAR 78-15536 and various NOAA Sea Grants to Rutgers University (NA79AA-D-00140 and NA81AA-D-00064), Woods Hole Oceanographic Institution (NA80AA-D-00077), and the University of Maine (NA81AA-D-00035). Contribution No. 1317 HPEL from the Center for Environmental and Estuarine Studies, University of Maryland. Publication No. NJSG-82-81 prepared under Project No. R/F-7 of NOAA grant NA81AA-D-00065.

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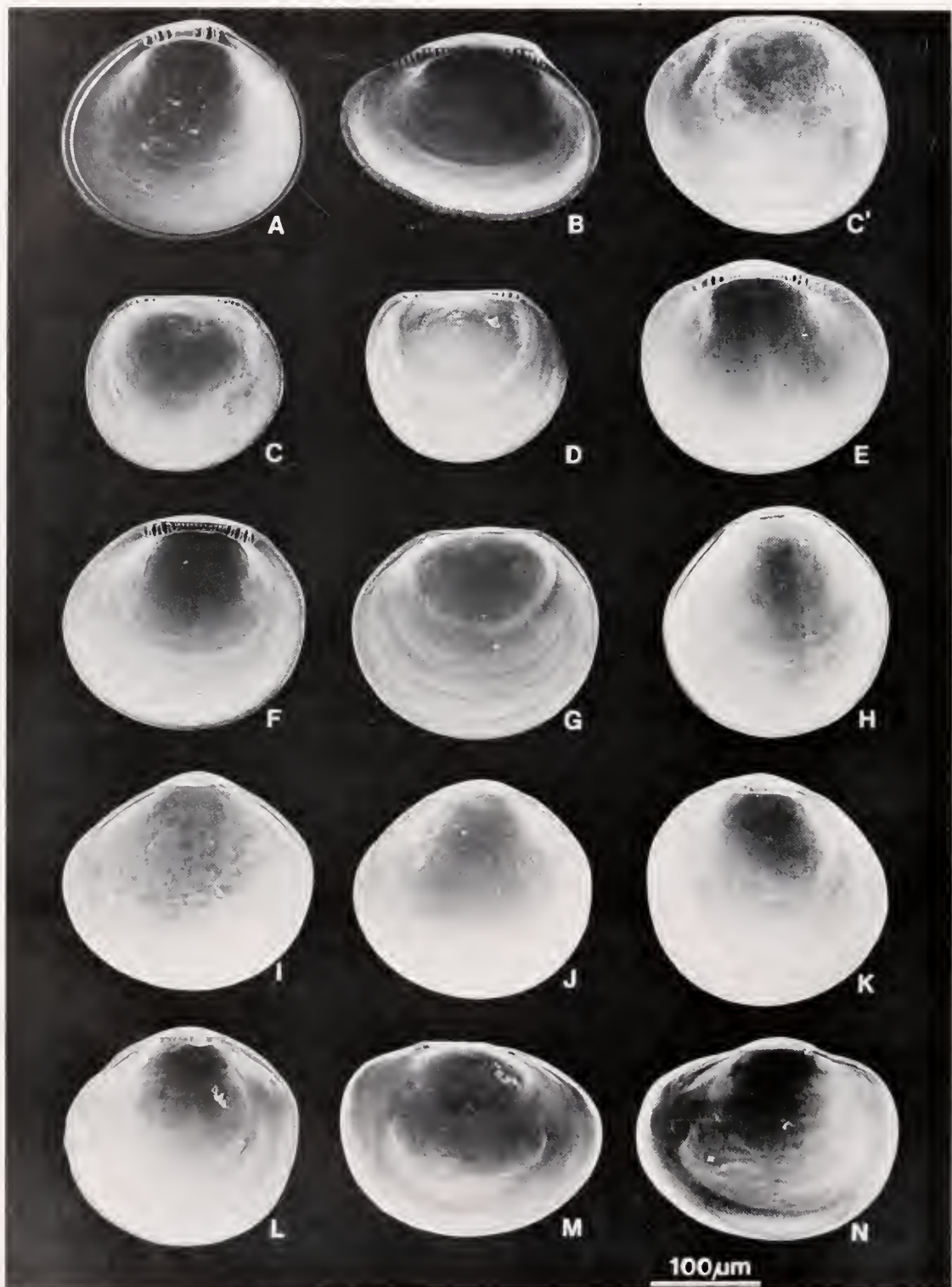


Figure 1. Scanning electron micrographs of disarticulated shell valves of planktonic larvae of various species of bivalve molluscs. A. *Crassostrea virginica* (right valve; mature larva). B. *Arca noae* (right valve; mature larva). C'. *Argopecten irradians* (right valve; mature larva). C. *Argopecten irradians* (left valve; straight-hinge larva). D. *Placopecten magellanicus* (left valve; straight-hinge larva). E. *Mytilus californianus* (left valve; mature larva). F. *Geukensia demissa* (right valve; mature larva). G. *Arctica islandica* (right valve; mature larva). H. *Mercenaria mercenaria* (right valve; mature larva). I. *Mya arenaria* (right valve; mature larva). J. *Mulinia lateralis* (right valve; mature larva). K. *Spisula solidissima* (left valve; mature larva). L. *Spisula solidissima* (right valve; mature larva). M. *Ensis directus* (left valve; mature larva). N. *Ensis directus* (right valve; mature larva).

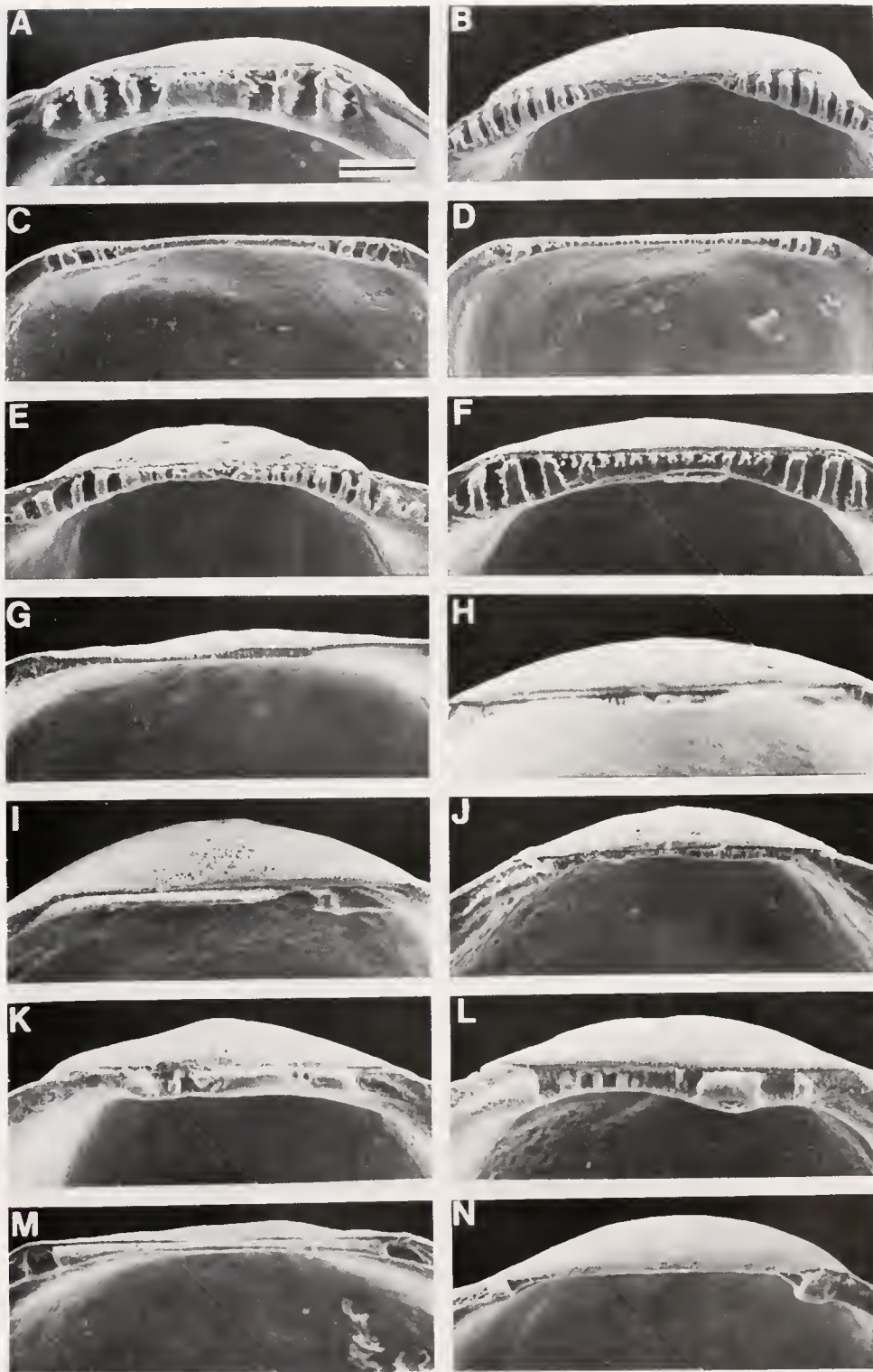


Figure 2. Scanning electron micrographs of the hinge region of the disarticulated shell valves of the specimens depicted in Figure 1. A. *Crassostrea virginica* (right valve). B. *Arca noae* (right valve). C. *Argopecten irradians* (left valve; straight hinge). D. *Placopecten magellanicus* (left valve; straight hinge). E. *Mytilus californianus* (left valve). F. *Geukensia demissa* (right valve). G. *Arctica islandica* (right valve). H. *Mercenaria mercenaria* (right valve). I. *Mya arenaria* (right valve). J. *Mulinia lateralis* (right valve). K. *Spisula solidissima* (left valve). L. *Spisula solidissima* (right valve). M. *Ensis directus* (left valve). N. *Ensis directus* (right valve). Scale bar (= 20  $\mu$ m) in A is applicable to all micrographs in this figure.



Figure 3. Scanning electron micrographs of disarticulated shell valves of straight-hinge larvae of three species of bivalve molluscs (*Geukensia demissa*, *Crassostrea virginica*, and *Arctica islandica*). Scale bar: 30  $\mu$ m).

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## MARKING STUDIES ON THE RED CRAB *GERYON QUINQUEDENS* SMITH OFF SOUTHERN NEW ENGLAND

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**ABSTRACT** During red crab tagging studies in upper continental slope waters off southern New England in May–June 1974, in depths of 275 to 1,100 m, 7,822 trap-caught red crabs (81 to 154 mm carapace width) were tagged with tags of vinyl tubing tied around the carapace. This type of tag is lost at molting. Through June 1981, a total of 593 recaptures were reported, mostly by red crab trap fishermen. Three recaptures were made as late as early 1981. The principle indicated movements were along the slope with maximum distances moved of 90 km in easterly directions and 55 km towards the west. Most of the crabs move little, however; the majority of recaptures were within 20 km or less of release positions. Short movements up and down the slope of up to about 6 km, with depth changes of up to 500 m, were also noted. No migration pattern was apparent in any of the movements.

The tagged crabs apparently grew slowly because the intermolt period of those recaptured in 1980–81 was 6 to 7 years or more, indicating that the harvested segment of the population (i.e., male crabs  $\geq 114$  mm carapace width) consisted of old individuals. Some ovigerous tagged crabs were caught throughout the period of recaptures, possibly indicating that sperm stored from mating at the time of the last pretagging molt fertilized new masses of eggs for several years. A Peterson estimate of the fishing rate from tag returns suggested that a small number of fishing vessels could exert a significant mortality on market-size crabs.

### INTRODUCTION

The red crab *Geryon quinquequedens* occurs on continental shelf and upper continental slope areas from south of Nova Scotia to Brazil (Rathbun 1937). While some individuals are found in Gulf of Maine waters as shoal to 40 m, the bulk of the population resides in deeper areas of the outer shelf and upper slope. The presence of an extensive, concentrated, and potentially exploitable red crab population on the upper slope at depths of about 275 to 1,000 m from Georges Bank to off Cape Hatteras has been recognized since the late 1950's (Schroeder 1959, McRae 1961).

Information on this resource led to the development in 1973 of a small commercial trap fishery for the species on slope areas off southern New England, following a decline there in the catch of lobsters (*Homarus americanus* Milne-Edwards) (Gerrior 1981). In 1980, the red crab catch from this fishery was 2,500 metric tons (t). A small amount of red crab effort also began in slope waters off Delaware, Maryland, and Virginia in 1976. Reported red crab landings there in 1980 were 65 t, although this is likely an underestimate of actual catch.

Some efforts were undertaken to gather data on the size and extent of the red crab resource and its population characteristics (Meade and Gray 1973, Haefner and Musick 1974, Wigley et al. 1975). These included a tagging program funded by the New England Fisheries Development Program (NEFDP), a cooperative venture of the National Marine Fisheries Service (NMFS) and the fishing industry for aiding

the development of fisheries for underutilized species (Rathjen 1974).

The state divisions of marine fisheries in Rhode Island and Massachusetts took part in the tagging. The Rhode Island division, under contract to NEFDP, prepared a report covering the first year of tag returns (Ganz and Herrmann 1975). The present report summarizes the results of the tagging through June 1981.

### TAGGING METHODS

When the tagging was planned, red crabs were being fished only on upper slope grounds between Hudson and Block canyons, south of New England (Figures 1 and 2). This was the area chosen for tagging because directed fishing was necessary for the recovery of tagged crabs.

The tagging was done during three trips in May and early June 1974 aboard a chartered commercial red crab fishing vessel. Crabs were caught and tagged in an area bounded by 39°40'–40°00'N and 71°30'–72°00'W (Figure 2). The first tagging trip was carried out 16–18 May 1974; the second trip, 25–27 May; and the third trip, 3–5 June. The crabs tagged during trips 1 and 3 were caught and released in depths of 290 to 365 m; those from trip 2 were caught and released in depths of 275 to 1,100 m.

The method of fishing during these trips was the same as that used in the commercial fishery. The crabs were caught in rectangular traps set in trawls of about 50 traps

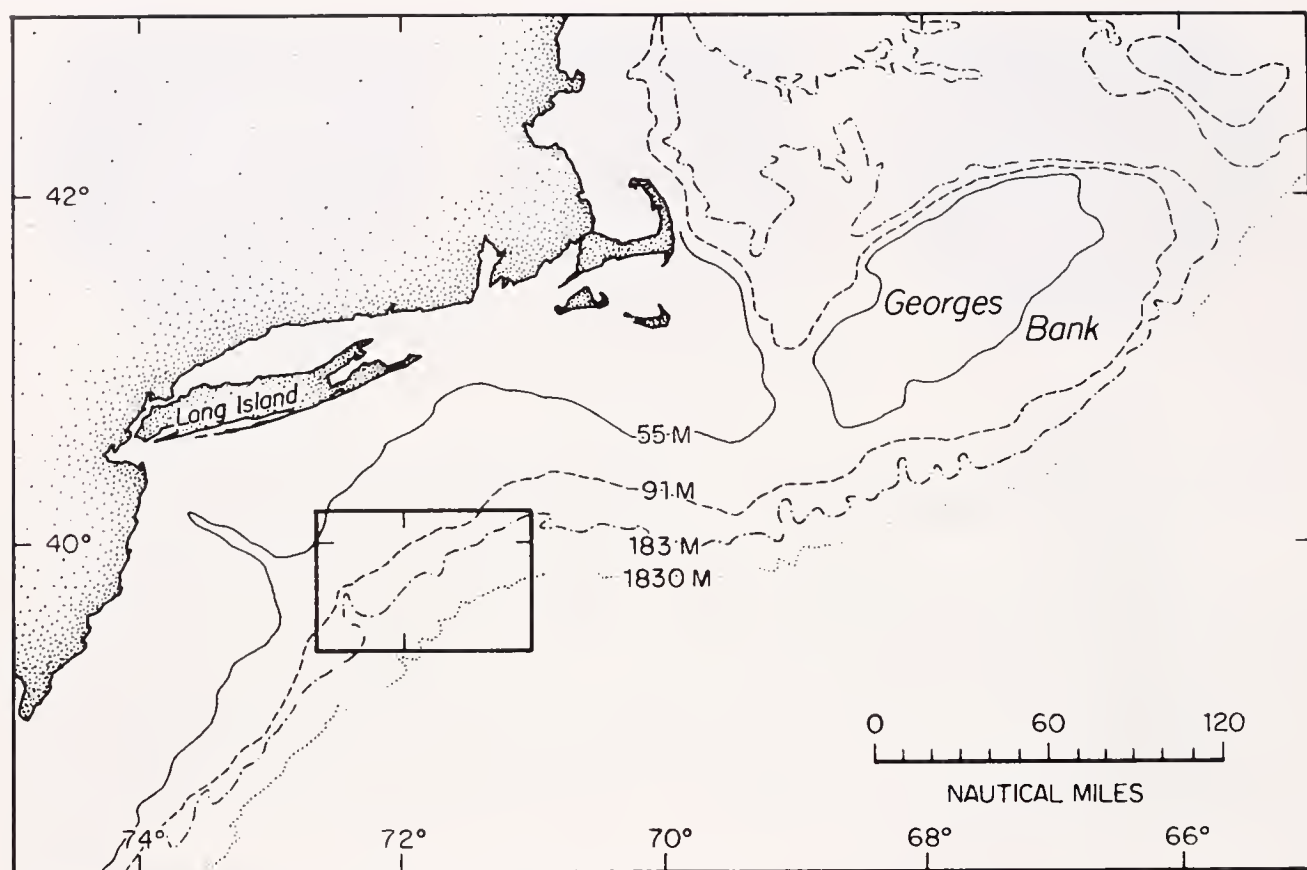


Figure 1. Bathymetric chart of grounds off southern New England including the general area (outlined) where red crabs were tagged in 1974.

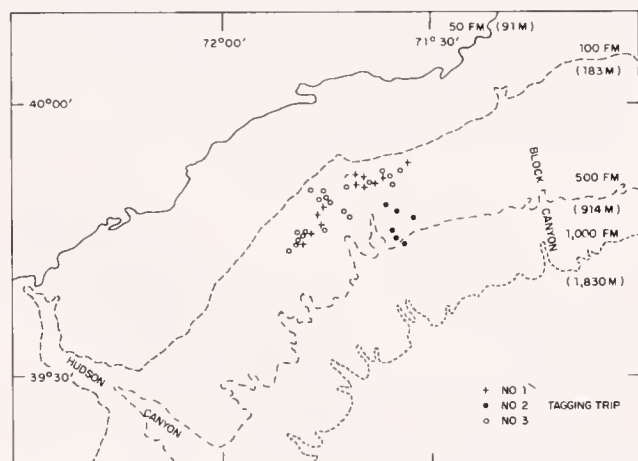


Figure 2. Chart showing the general area where red crabs were tagged in 1974 (see Figure 1) and the specific locations where groups of tagged crabs were released, shown by symbols, during each of the three tagging trips.

each. The traps were of wooden lath construction with a double parlor, a type commonly used by offshore lobster fishermen (Ganz and Herrmann 1975). Dimensions were 1.2-m long, 0.8 m wide, and 0.5 m deep.

Tagging was conducted around the clock by six taggers working in shifts of three men each. No attempt was made to select crabs for size or sex. When a trap was hauled all lively individuals in it were tagged. Male red crabs are larger than females and also are heavier for a given carapace width (Haefner 1978); fishermen, therefore, land only males and primarily those  $\geq 114$  mm (4.5 inches) carapace width, which are the sizes preferred by the market. Females and undersized males are discarded at sea. During the culling process the entire catch is handled; therefore, the discards receive about the same level of scrutiny for tags as the kept catch.

For each tagged crab the maximum carapace width (mm), measured with calipers from tip to tip of the fifth lateral spines, and sex were recorded. Information on crab condition, such as injuries or missing legs and claws, was routinely recorded. In addition, notes on some of the ovigerous crabs were made.

The tags, of yellow vinyl tubing 1.7 mm in diameter (Watson 1970), were attached to the crabs by simply tying them loosely around the carapace (Figure 3). They were individually numbered and bore return instructions. Because this type of tag was lost at molting, tag returns were only from crabs that had not molted between tagging and recapture.



Figure 3. Red crab with vinyl tag in place around carapace.

Posters bearing tagging information and instructions to tag finders were posted in ports of landing. A reward of \$2.00 was paid for a tag with the crab and \$1.00, for a tag only. Because the landed value of a market-size crab averaged only \$0.50, there was some monetary incentive for returning crabs as well as tags.

Tag recoveries were mostly from commercial red crab fishing vessels which varied in number from one to four in New England waters in 1973–1981. Some tags were also obtained by lobster fishermen who trap lobsters in the upper slope areas a little shallower than those generally fished for red crabs.

The returned tags and crabs were turned in to NMFS port agents who collected data on the catch. They recorded recovery location, depth, recovery date, and other available data on tag return forms and forwarded these to the NMFS Northeast Fisheries Center at Woods Hole. A few additional tags were mailed in by fishermen. In cases where the crab as well as the tag were returned, the crab was measured, and sex and shell condition were noted. Except for the 1975 recaptures, females were checked for eggs.

An attempt was made to obtain recovery locations for all recaptures. For 60% of the tags returned, however, the general vicinity fished during that particular trip was the best information available. Because vessels commonly covered a strip of slope 10 miles long or more in the course of a trip, these tag returns were of limited use for measuring crab movement.

## RESULTS AND DISCUSSION

### *Sizes of Tagged and Recaptured Red Crabs.*

In the three 1974 sampling trips combined, 7,822 red crabs were tagged. The percentage size frequencies of those tagged in each trip are presented in Figure 4. The range in carapace width of males was 81 to 154 mm, and of females, 85 to 139 mm (Table 1). The first and third tagging trips were conducted in depths of 290 to 365 m and over the

areas more frequently fished by commercial red crab vessels. Crabs from these trips, therefore, were similar in size composition to commercial catches before culling of discards. On the second tagging trip most of the crabs were caught in about 530 to 1,000 m depths (Figure 2), which was deeper than normally fished commercially. These crabs were smaller than those from the other two trips (Figure 4, Table 1). The mean carapace width of males in trips 1 and 3 combined was 124.7 mm, and that of females, 110.4 mm. In trip 2, the mean sizes were 118.4 mm for males and 104.6 mm for females. This decreasing size with increasing depth agreed with the general finding that there are more smaller red crabs in deeper water (Wigley et al. 1975).

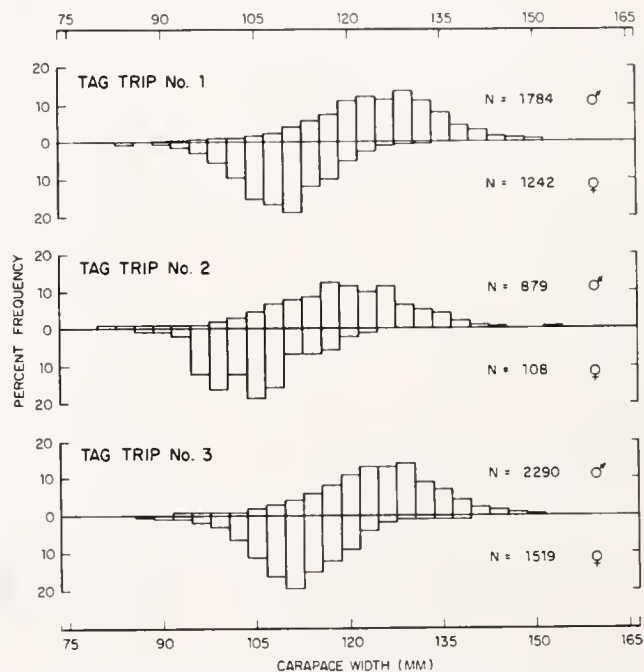


Figure 4. Percentage size frequencies and total numbers of male and female red crabs tagged during each of the three 1974 tagging trips.

The absence of crabs < 81 mm carapace width among those tagged appeared to be due to those smaller sizes seldom entering traps, at least in the areas of tagging. However, red crabs much smaller than 81 mm have been caught on the slope areas in otter trawls (Haefner and Musick 1974, Wigley et al. 1975).

The ratio of males to females during trips 1 and 3 was similar at 1.44 and 1.51, respectively (Table 1). In trip 2, however, the males outnumbered the females by more than 8 to 1. Few female red crabs were caught beyond depths of about 650 m, a distribution pattern which agreed with the findings of Ganz and Hermann (1975), Wigley et al. (1975), and Haefner (1978).

Through June 1981, 593 tagged crabs were recaptured (7.58% of those released). The percentage of males recaptured was 8.14%, and that of females, 6.62% (Table 2). The

TABLE 1.

Numbers of male and female red crabs tagged, mean and range of carapace width, and sex ratio for each tagging trip and all trips combined, May–June 1974.

Trip Number	Males			Females			Sex ratio male/female
	Number of Crabs	Carapace width (mm)		Number of Crabs	Carapace width (mm)		
		Mean	Range		Mean	Range	
1	1,784	124.9	90–149	1,242	109.2	85–133	1.44
2	879	118.4	81–154	108	104.6	87–122	8.14
3	2,290	124.5	92–150	1,519	111.3	86–139	1.51
All trips	4,953	123.6	81–154	2,869	110.1	85–139	1.73

TABLE 2.

Numbers of tag recoveries, mean carapace widths (mm), percentages recaptured<sup>1</sup>, and sex ratios for each year, 1974–1981, for tagged male and female red crabs in 1974 releases.

Year	Males			Females			Sex ratio male/female
	Number	Mean Width	% Recaptured	Number	Mean Width	% Recaptured	
1974	160	127.3	3.23	107	110.7	3.72	1.50
1975	75	128.2	1.51	45	109.2	1.57	1.67
1976	6	127.0	0.12	3	105.0	0.10	2.00
1977	137	125.5	2.77	24	111.2	0.84	5.71
1978	1	133.0	0.02	0	—	—	—
1979	13	129.8	0.26	2	97.5	0.07	6.50
1980	10	129.6	0.20	7	112.0	0.24	1.43
1981 <sup>2</sup>	1	140.0	0.02	2	111.5	0.06	0.50
All years	403	127.0	8.14	190	110.2	6.62	2.12

<sup>1</sup>Percentage of original number tagged.

<sup>2</sup>January–June 1981.

numbers of tagged crabs recaptured varied greatly from year to year, depending on level of fishing effort in the general vicinity of tagging. In 1976 and 1978, for example, there was little fishing effort in the release areas; consequently, there were few tag recoveries (Table 2).

It was evident that molt frequency was low because some of the tagged crabs had not molted for nearly 7 years. If it is assumed that tagged crabs molted with the same frequency as untagged ones, a slow growth rate is indicated. Further information on this is provided by the mean sizes of recaptures in each year (Table 2). These data show that there were little differences in mean size at recapture with the passage of time. For example, the mean carapace width of all male crabs tagged was 123.6 mm (Table 1), while that of the 10 males recaptured in 1980 was 129.6 mm, not a great deal larger than at tagging. The situation was similar for females: those tagged had a mean carapace width of 110.1 mm and the seven recaptures in 1980 had a mean width of 112.0 mm (Tables 1 and 2).

Additional data on the size of recaptured crabs compared with size at tagging were provided by size frequencies of recaptures in 1974, 1975, and 1977, from releases of tagging trips 1 and 3, the trips from which most of the returns were obtained (Figure 5). These data showed that the size

composition of recaptured crabs of both sexes in those years did not differ greatly from that of the tagged population, further indicating long intermolt periods. There was, however, a general lack of the smallest sizes among the recaptures, leaving the possibility that many of these had molted before they could be recaptured.

The comparative rates of tag returns by sex from 1974 to 1981 provided some indication that the tagged females may have molted sooner than the males (Table 2). The rate of return was similar for both sexes in 1974 to 1976, with a mean male-to-female ratio of approximately 1.55 among the returns. In 1977, however, this ratio was 5.71, suggesting that by this later year there was a greater loss of tags to molts among females. In 1980–81, the sex ratio shifted back toward females again, although the total number of returns in those years was small (Table 2). In view of that and of the patchy nature of red crab distribution by sex (Wigley et al. 1975, Haefner 1978), the observed sex ratio variations may be of rather limited use for estimating frequency of molt.

As a result of tagging studies of male snow crabs (*Chionoecetes opilio* [O. Fabricius]) with tags like those used for red crabs, Watson (1970) reported that recaptures were obtained for up to three years after tagging, thus

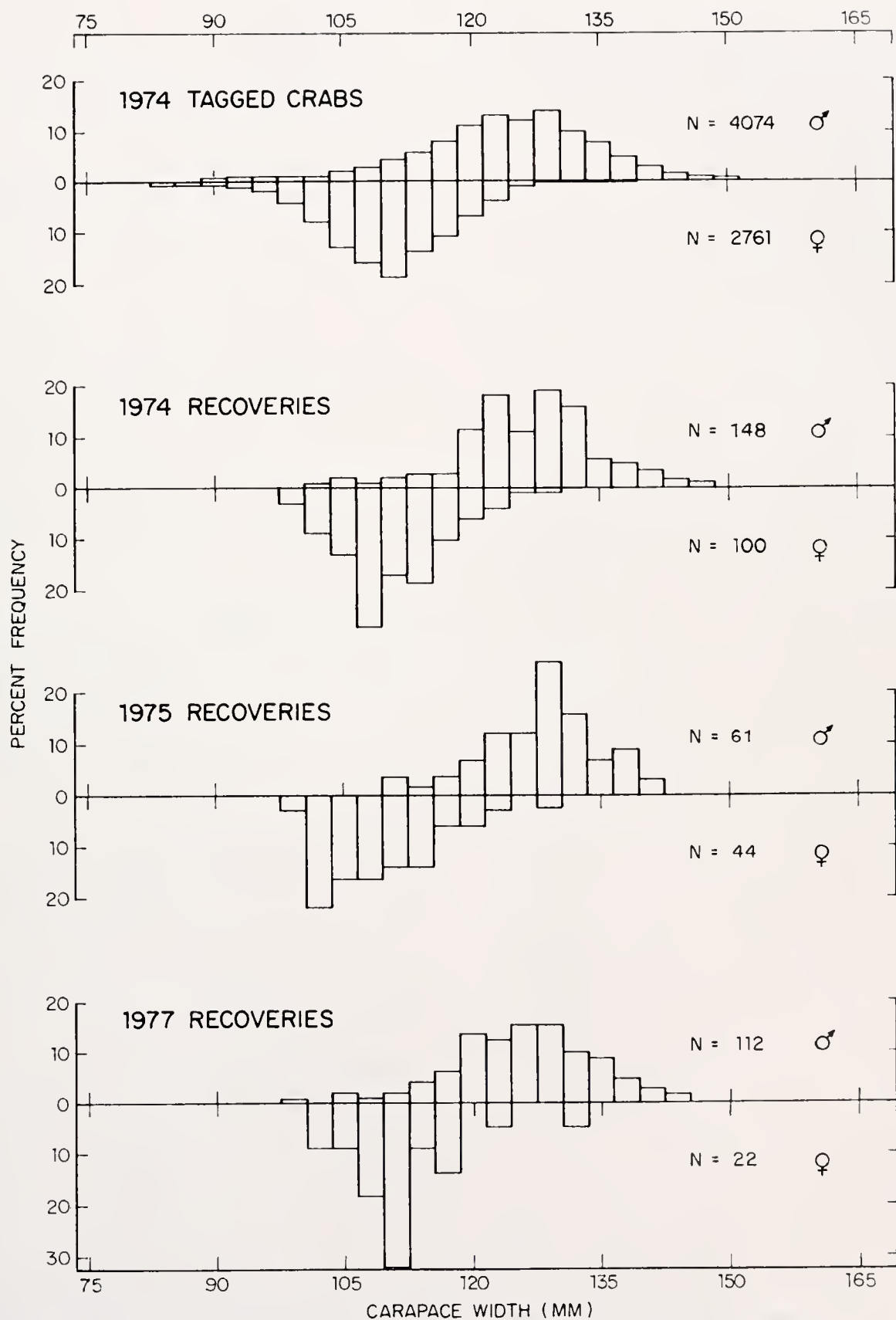


Figure 5. Percentage size frequencies and total numbers of male and female red crabs tagged in 1974 in tagging trips 1 and 3, and of those recaptured in 1974, 1975, and 1977, from those releases.

indicating a relatively long intermolt period in this species also. The snow crab lives in water temperatures of about  $-1^{\circ}$  to  $2^{\circ}\text{C}$  (Imbeault 1969) which is colder than the environment of the southern New England red crab where upper-slope bottom temperatures run from about  $4^{\circ}$  to  $8^{\circ}\text{C}$  (Schroeder 1959, Wigley et al. 1975, Haefner 1978).

#### *Tagging Mortality.*

Information on tagging mortality is useful for interpreting results in tagging studies. While the extent of this is unknown for the tagged red crabs, two tag return facts led us to believe that it was not great. The first relates to recoveries immediately following tagging. During the three tagging trips, 118 of the tagged crabs were recaptured, some within a day of being tagged and released. Because crabs had to move into baited traps to be recaptured, these recaptures soon after tagging indicated that recovery from being caught and tagged was rapid and that tagging shock was minimal. Of the 118 tagged crabs recaptured during tagging operations, 112 were in good enough condition to be returned to the water. The percentage of these subsequently caught by fishermen (6.6%) was similar to, though somewhat lower than, the overall tag recapture rate of 7.58%, thereby suggesting relatively low mortality from catching and handling.

The other source of information on tagging mortality was from observations of injured crabs that were tagged. Data from the tagging logs indicated that many of the crabs caught for tagging had one or more appendages (claws or walking legs) missing. It is likely that, in most cases, these were lost during catching and tagging operations because crabs, crowded in traps, frequently closed their claws on each other, and the resulting injuries sometimes caused autotomy of affected appendages. Of the 7,822 crabs tagged and released, 1,658 (21.2%) had missing appendages. Most of those had lost only one or two appendages, but some had lost three or four.

The return rate of tagged crabs with missing appendages was examined for two periods: 1974–76 and 1977–80. In 1974–76, 19% of the 396 returned crabs had appendages missing at tagging. This figure is only slightly lower than the 21.2% for all tagged crabs. The possible explanations for the lower rate of recapture of these injured crabs include somewhat higher mortality and reduced mobility because of limb loss. The conclusion from this, however, is that in 1974–76 mortality was not markedly greater in injured crabs, indicating low mortality from capture and tagging procedures.

Only 8.8% of the 194 tagged crabs returned in 1977–80 had missing appendages at tagging, a considerable decline from the 21.2% with missing appendages of all releases. Causes for this marked change, while unknown, may be related to earlier molt and, therefore, accelerated tag loss among crabs with lost appendages. However, a delayed tagging mortality among injured crabs cannot be ruled out.

#### *Movements of Tagged Crabs.*

Of the 593 tagged crabs recaptured through June 1981, 547 (92.2%) were caught in red crab traps and 44 (7.4%) in offshore lobster traps. The other two recaptures (0.3%) were by a research vessel otter trawl.

Red crab fishing effort in the years following tagging, based on interviews with crab fishermen, covered the upper slope area in depths of about 290 to 750 m from southwest of Hudson Canyon to off southern Georges Bank. As indicated below, the effort and area of operations varied considerably from year to year.

In the first few years of the fishery, New England red crab vessels changed fishing areas with some frequency. The principle reason given was that the catch of market-size crabs declined after a period of fishing. Fishermen moved to another area when that occurred or sometimes before. In more recent years, fishermen have routinely moved their gear each trip, thereby minimizing that problem (Gerrior 1981). These patterns of fishing and the intermittent nature of the fishery in early years have contributed to the irregular distribution of red crab fishing effort. That irregular distribution was reflected in the tag return rates from year to year.

Fishing effort by offshore lobster vessels from 1975 to 1980 was distributed over the entire outer shelf area from southern Georges Bank to off Virginia. Those vessels fished out to depths of about 310 m, which is the upper fringe of red crab distribution in that area. Some lobster trapping occurred on those grounds in all years following tagging, although the intensity varied widely from place to place.

Only returns with accurate positions were plotted on charts showing recapture locations of tagged red crabs (Figures 6–8).

In 1974, 269 tags were returned; 236 were from red crab vessels, 31 from lobster vessels, and 2 from a research vessel. All tagged crabs were recaptured in the general area of release which was where all 1974 post-tagging trapping effort occurred (Figure 6). Some crabs moved in either an easterly or westerly direction along the slope from the release points with little indication of a directed movement. The maximum apparent distance moved was about 40 km, but most crabs exhibited little or no movement.

All 1974 recaptures were made in August and September, the only post-tagging period of 1974 in which red crab fishing took place. Most of the tag returns in 1974 were not plotted on Figure 6 because of imprecise recapture locations. However, because all 1974 post-tagging effort was in the general tagging area, the recaptures not plotted were caught in the same vicinity as those shown on Figure 6.

In 1975, 118 tagged crabs were returned: 116 from crab vessels and 2 from lobster vessels; in 1976, only 9 tagged red crabs were recaptured: 1 from a crab vessel and 8 from lobster vessels (Figure 7). Most 1975 recaptures were from the tagging area. However, there was a considerable scattering

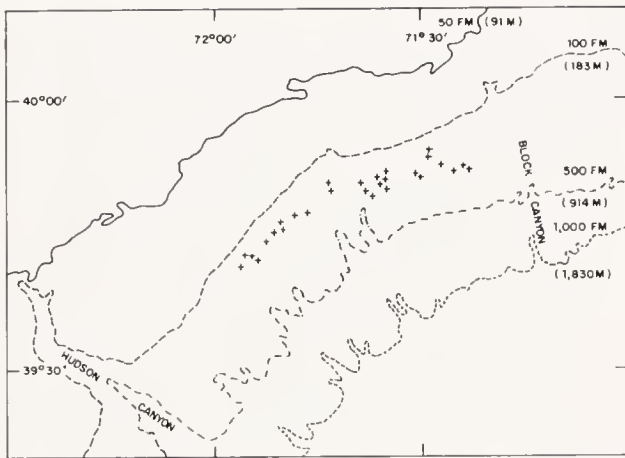


Figure 6. Recapture locations (X) for red crab tag returns in 1974 from all 1974 releases. (Only returns with accurate recapture locations are plotted.)

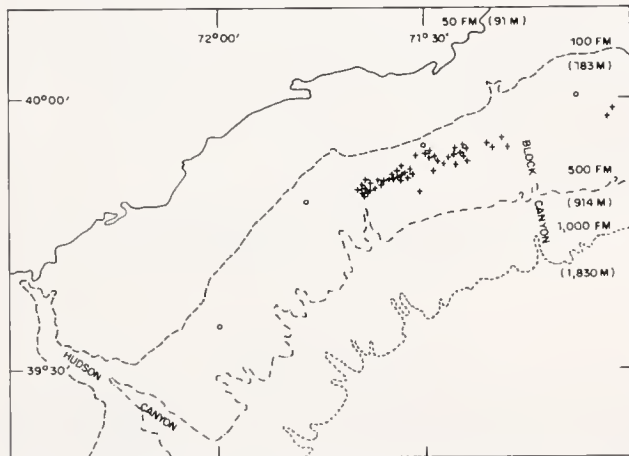


Figure 7. Recapture locations for red crab tag returns in 1975 (X) and 1976 (O) from all 1974 releases. (Only returns with accurate recapture locations are plotted.)

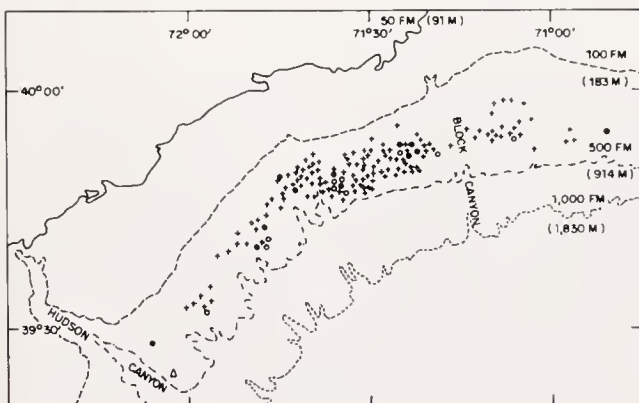


Figure 8. Recapture locations for red crab tag returns in 1977 (X), 1978 (Δ), 1979 (●), and 1980 (○) from all 1974 releases. (Only returns with accurate recapture locations are plotted.)

of crabs eastward, with a maximum indicated movement of about 65 km. Fishing for red crabs in 1975 covered the eastern part of the tagging area (Figure 2) and slope areas to about 130 km to the east of the tagging area.

All of the 1975 tag recaptures by red crab vessels occurred in October–December, when there was an active fishery in the eastern part of the release area. The two returns from lobster vessels were caught in March within a few kilometers of points of release.

In 1976, the red crab effort was entirely to the east of 71°10'W, and 8 of the 9 tagged crabs were caught by lobster vessels, from March to July, in the general area of the 1974 releases (Figure 7). The ninth was caught by a red crab vessel in June about 85 km east of its release point.

In 1977, 161 tagged crabs were returned: 159 from red crab vessels and 2 from lobster vessels. In 1978, only one crab was recaptured, while in 1979 and 1980, there were 15 and 17 recoveries, respectively; all of the 1978–1980 recaptures were by red crab vessels.

Most of the recaptures in 1977 were made within a few kilometers of the tagging locations; however, there was some movement of tagged crabs both east and west along the slope from the release points (Figure 8). Recoveries were made throughout the year, but most were caught in April–June. In 1978, the single recaptured crab was caught in January along the eastern side of Hudson Canyon about 45 km to the west of the release point (Figure 8).

In 1979, most of the returns were from the general vicinity of tagging; however, one crab each was recaptured about 90 km east of and 55 km west of their respective release points (Figure 8). Four returns were caught in April, three in July, seven in September, and one in December.

In 1980, the pattern of return locations of the 10 recaptures with accurate return positions was similar to that for 1979: the recaptures were dispersed over the entire area between 71°00'W and 72°00'W (Figure 8). Nine of the 1980 returns were caught in January–March, five in June, and three in November.

Fishing effort for red crabs in 1977 extended over the entire tagging area as well as slope areas to about 150 km east and 35 km southwest. Considerable effort was expended in the Block Canyon region where many of the 1977 recaptures were obtained (Figure 8). In 1978, the crab effort covered slope grounds southwest of the tagging area (from about 72°00'W to 72°40'W) and from 120 to 425 km east (about 65°50'W to 70°00'W). There was no directed effort within the vicinity of tagging, and only one tagged crab was recovered (Figure 8). The 1979 crab effort covered the tagging area to about 55 km southwest (near Hudson Canyon), and as far as 185 km east (about 68°40'W). In 1980, the effort was from Hudson Canyon to slope areas about 220 km north and east, with most of the effort falling within the eastern part of the tagging area, just southwest of Block Canyon.

There were three tag recaptures through June in 1981 (Table 2). While the recovery locations for these are not shown on Figure 8, all were caught on slope areas within 30 km of release positions. One was caught in March in a lobster trap; the other two were taken in April in red crab traps. Red crab effort in the first half of 1981 was mainly east and west of the tagging area; however, there was a small fishing effort within the tagging area in April.

The number of tag recaptures in relation to the estimated red crab catch within the tagging area has declined greatly over the years. Losses of tags to molts, natural mortality, and tag detachment, as well as fishing mortality, have all contributed to the reduction of the tagged population; therefore, we expect only a few tag recaptures from these experiments in the future.

The pattern of recapture locations for all years indicated that most tagged crabs were recaptured within about 20 km or less of where they had been released. Maximum movements were about 90 km to the east and 55 km to the southwest of the release points. None of the tagged crabs were recaptured on the southwestern side of Hudson Canyon. The movement of crabs seemed to be more of dispersal along the slope rather than a directed movement. Movement may have been greatest in an easterly direction, but the heaviest concentration of red crab fishing effort was also east of the tag-release centers.

In addition to the movements along the slope, some tag returns indicated that movements of about 1 to 6 km up or down the slope had occurred resulting in depth changes of up to 500 m. While such movements may be related to the red crab spawning cycle, there was no detectable seasonal migration pattern in these observations.

In the tagging study of snow crabs in the Gulf of St. Lawrence, Watson (1970) found that most of the tagged crabs had moved only a few kilometers in the first 2 years with movements of up to 52 km in the third year. As in red crabs, the movements appeared to be nondirectional.

#### Cycle of Egg Production Based on Tagging and Recapture Data.

Newly laid eggs of the red crab (early stage) are red or orange, partially developed ones (middle stage) are brown, and those approaching hatching (late stage) are nearly black (Ganz and Herrmann 1975). The color changes result from the gradual absorption of yolk material and the formation of dark eye pigment and other larval characters; they are similar to those described for developing eggs of the blue crab (*Callinectes sapidus* Rathbun) (Van Engel 1958). Information obtained aboard commercial red crab vessels by Ganz and Herrmann (1975) indicated that, in general, ovigerous crabs had early-stage eggs in summer and fall, middle stage in winter, and late stage in spring, suggesting spring hatching and summer and fall egg deposition. This seasonal cycle is similar to patterns tentatively proposed by Wigley et al. (1975) and Haefner (1978) who suggested, on the basis of research vessel collections of ovigerous red

crabs, that a high incidence of hatching occurred between January and June. Haefner (1978) found few ovigerous crabs in June and many in November and January.

We found evidence of a pattern similar to those described above. In the May–June 1974 tagging trips, 27 ovigerous crabs were reported among those tagged. While it is likely that other ovigerous crabs were overlooked at tagging, the log notes on these 27 crabs were of interest. The eggs of 21 crabs were “black” and/or “eyed,” and they probably were late stage. One of the other six had early-stage eggs and five had no clear indication of stage.

Records of egg stages of ovigerous crabs recaptured from 1974 to 1981 provided further information on the reproductive cycle (Table 3). The eggs of these were classed as being in the early, middle, or late stages of development, using the color criteria given above (Ganz and Herrmann 1975). Because color is not completely reliable for determining stage (Haefner 1978), we also used a microscope to examine the eggs from 1977 to 1981 returned crabs; in some cases the embryos were removed to check development. These data showed that among the recaptured crabs those caught in late summer had early-stage eggs and those caught in spring mostly had late-stage eggs (Table 3).

TABLE 3.

Numbers of ovigerous tagged red crabs recaptured in 1974–1981<sup>1</sup> and month(s) caught, by stage of egg development. (Except as noted, crabs were nonovigerous when tagged.)

Year	Stage of Egg Development				Total
	Early	Middle	Late	Unknown	
1974	13 (Aug–Sep)	1 (Aug)	—	2 (Aug) <sup>2</sup>	16
1977	4 (Sep)	1 (Apr)	5 (May)	—	10
1979	1 (Sep)	—	—	—	1
1981	—	—	1 (Apr)	—	1

<sup>1</sup>Crabs recaptured in 1975 were not examined for eggs.

<sup>2</sup>One of these was ovigerous with early stage eggs when tagged in May 1974.

The above observations, while not covering the entire year, indicated that red crab hatching occurred primarily in spring and egg laying in summer and fall, coinciding reasonably well with findings of Ganz and Herrmann (1975), Wigley et al. (1975), and Haefner (1978). Whether or not inseminated females may lay fertile eggs in every year for several years after mating is unknown. These data show, however, that eggs may be laid some years after a molt and, assuming that mating occurs only right after a molt, that stored sperm remains viable for several years.

With respect to crab mating, Watson (1972) found that some of the immature female snow crabs from the Gulf of St. Lawrence which were held in aquaria mated within minutes after molting to maturity and then laid fertile eggs within 24 hours. In the same study, a number of

ovigerous females with late-stage eggs hatched their eggs in aquaria and, then without molting or contact with a male, laid new fertile eggs within about 2 weeks. A similar pattern may occur in red crabs with immature females mating after the molt that brings them to maturity and mature females mating only after a subsequent molt.

No direct information on red crab mating was obtained from the tagging study. However, there have been some underwater observations in relation to mating. During dives on 24–29 June 1975 in Veatch Canyon (approximate position 39°52'–40°00'N, 69°36'–69°38'W, 275 to 1,255-m depth) with the research submarine *Alvin*, numerous paired red crabs were observed, photographed, and videotaped by NMFS scientists (J. R. Uzmunn, NMFS, NOAA, Northeast Fisheries Center, Woods Hole, MA 02543, personal communication). In those cases the male was apparently holding the female in a pre- or post-molting embrace, as is common in brachyurans. It seems likely that the females molted just before mating took place, although hard-shell mating also occurs in some species (Hartnoll 1969). These observations, although they did not define the entire season of mating, indicated that there was considerable mating activity in June in that area. Summer mating, followed soon after by egg laying, would fit in with the cycle of egg development described above.

#### *Fishing Mortality.*

As mentioned above, fishermen have reported decreases in the catch of market-size male red crabs in an area following a period of fishing, indicating that exploitation can exert a significant mortality on the marketable segment of the population. If such an area is not fished for a time, the proportion of large males again rises, presumably through growth and, possibly, migration, and more profitable fishing may be resumed. While data from long-term tag returns may be used to estimate this fishing mortality rate, the scattered and sporadic nature of red crab fishing effort in the years following tagging makes this impractical. A further complicating factor is that the tagged population was continually being reduced by an unknown amount through molts and the consequent loss of tags.

Thus, the best means for obtaining any measure of fishing mortality in the tagging area appeared to be from early recaptures, before large tag losses had occurred. We, therefore, used the recaptures in late 1974 for estimating the rate of exploitation at that time.

The post-tagging fishing effort in 1974 did not begin until mid-August, or more than 2 months after tagging, which gave the tagged crabs time to become dispersed in the general tagging area. Fishing effort consisted of only 3.25 red crab trips by a single vessel between 17 August

and 23 September 1974. (The fractional trip resulted from low market demand.) The catch from those trips approximated 117,000 market-size males and about 107,000 females and undersized males that were discarded for a total catch of 224,000 crabs.

(Live weight of red crab landings was obtained by multiplying the weight of butchered crab sections, the landed form, by 1.76, the NMFS butchered-to-live conversion factor. The estimated number of market-size crabs was obtained by dividing landed live weight in kilograms by 0.64 kg, the mean weight of a market-size male red crab in tagging trips 1 and 3, which were in areas fished commercially; the mean weight was determined from mean carapace width using a carapace size-to-weight relationship from Haefner (1978). The estimated numbers of discards were based on the proportion of market-to-discard crabs in the catches of tagging trips 1 and 3.)

During those 3.25 trips 230 tagged crabs were recaptured from the 7,822 released, indicating a tag return rate of 2.9%. If all crabs caught were landed, the 2.9% could be used directly as a Peterson estimate of the fishing rate (Ricker 1975) for these 3.25 trips in the tagging area. Only about 52% of the catch was saved, however, and the discarded 48% was assumed to survive (for calculation purposes). The estimated fishing rate on the market-size crabs, therefore, was 1.51% ( $0.52 \times 2.9\%$ ), and the fishing rate per trip was 0.45% ( $1.51\%/3.25$ ). A vessel of the size that caught the 230 tagged crabs can make about 50 trips per year and, at the 1974 level of vessel efficiency, could exert an estimated annual mortality on market-size red crabs in the tagging area of 23% ( $50 \times 0.45\%$ ). Because there was likely some unknown discard mortality, tagging mortality, and loss of tags to molts between tagging and recapture, this may underestimate total fishing mortality. However, a nonrandom distribution of the tagged crabs in the tagging area, a likely circumstance, could lead to an overestimate of total fishing mortality.

The above calculation is for the situation where fishing takes place in the same general area for an entire year. In the actual case fishermen routinely change areas. Despite the obvious shortcomings of this estimate, it is of value for projecting what the fishing rate on male crabs might be in relation to stock abundance, estimated by Wigley et al. (1975), as the level of fishing effort increases.

#### ACKNOWLEDGMENTS

For their help in this study we thank William Whipple, who made the vessel *Mars* available for the tagging work; the captain and crew of the *Mars*; and Thurston Burns, James Costakes, Warren Handwork, Raymond Heines, James Herrmann, Clifford Newell, Kenneth Pecci, and Ronald Smolowitz, all of whom assisted with the tagging operations.

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ABSTRACTS OF TECHNICAL PAPERS

*Presented at 1981 Annual Meeting*

NATIONAL SHELLFISHERIES ASSOCIATION

Williamsburg, Virginia

August 2–6, 1981



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# CYTOGENETIC AND ALLOZYMIC CONFIRMATION OF INDUCED POLYPLOID IN CLAMS AND OYSTERS

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Triploid shellfish may be useful in aquaculture. To confirm the efficacy of treatments meant to induce triploidy, however, a rapid method is needed to screen large numbers of animals. Two groups of oyster (*Crassostrea virginica*) zygotes were treated with 0.5 mg/l cytochalasin B in 1979; group 1 was treated from 0 to 15 minutes after fertilization, and group 2 was treated from 15 to 30 minutes after fertilization. Chromosomal analysis showed 54% of group 1, and 85% of group 2 were triploid. Electrophoretic analysis of enzymes coded by the biochemical loci PGM, PGI, EST-1, EST-3, and 6PG revealed 50% of group 1, and 77% of group 2 were triploid. Two groups of soft-shell clam (*Mya arenaria*) zygotes were treated with 0.5 mg/ml cytochalasin B in 1980; group 1 was treated from 0 to 15 minutes after fertilization, and group 2 from 15 to 30 minutes. Electrophoretic analysis for 6PG, EST-3, and PGM revealed 85% of group 1, and 78% of group 2 were triploid. Diploid clams had a 2N chromosome complement, previously unreported, of 34; triploid clams had 50 chromosomes. The starch-gel electrophoresis was an efficient, fast, and valid method for screening large numbers of shellfish for polyploidy.

# MANAGEMENT OF A HYDRAULIC-ESCALATOR HARD CLAM FISHERY IN SOUTH CAROLINA

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A commercial fishery for hard clams (*Mercenaria mercenaria*) using hydraulic-escalator dredges has been successful in the Santee Delta, SC, for 8 years. Located 72.4 km northeast of Charleston, the Santee Estuary is the fourth largest drainage system along the Atlantic coast, south of the St. Lawrence River. The extensive deltaic plain is characterized by over 8,000 ha of impoundments, fluctuating salinities (0.02 to 36 ppt), and two major distributaries, the North and South Santee rivers.

Hydraulic-escalator clam harvesters were introduced into the Santee Delta during early 1974, based on patent tong survey results in 1973, that indicated high-density clam populations were overburdened by subtidal oysters (*Crassostrea virginica*) and shell. Following an environmental

impact evaluation of the mechanical harvesters in this pristine area, permits were issued to seven resident vessels. A procedure of alternating harvest areas each year, and restricting harvesting to 2 days/week have allowed the standing crop to remain viable through natural recruitment. Currently the fishery operates from January to mid-April—predominantly regulated by ex-vessel clam prices. Since the beginning of the fishery in 1974, over 28 million clams have been harvested and marketed in ungraded bags. Rotation of harvest, incidental oyster catch, operating efficiency, and aspects of managing the renewable resource are discussed.

# RELATIONSHIPS BETWEEN THE PARAMETERS OF THE VON BERTALANFFY GROWTH FUNCTION AMONG BIVALVES AS REVEALED WITH THE AUXIMETRIC GRID

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The von Bertalanffy growth function (VBGF) describes growth using two parameters:  $L_{\infty}$  (or  $W_{\infty}$ ), the asymptotic length (or weight); and  $k$ , the growth constant. Pauly's auximetric grid, where  $\log k$  is plotted against  $\log W_{\infty}$ , was adapted to examine the relationship between  $k$  and  $L_{\infty}$ . These two parameters are shown to be negatively correlated. The distance between any point on the grid and the base line corresponds to the logarithm of Gallucci and Quinn's omega parameter ( $\omega = k \cdot L_{\infty}$ ) for the VBGF. It is suggested that  $\omega$  is a better indicator of the rate and potential of somatic production than is  $k$ . Plots of  $k$  and  $L_{\infty}$  are localized between different species and between different families. Omega appears to increase with increasing depth. This consistency of observations suggests an underlying environmental control for the pattern of growth.

# VARIATIONS IN THE LIFE HISTORY PARAMETERS OF POPULATIONS OF THE SOFT-SHELL CLAM *MYA ARENARIA* (LINNÉ) ALONG A LATITUDINAL GRADIENT

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Environmental and biological data were collected from 25 populations of the soft-shell clam *Mya arenaria* (Linné) located throughout the latitudinal range of the species. Principal components analysis was used to characterize

habitat variability. This analysis showed that many environmental parameters (e.g., temperature, salinity, sediment grain size, tidal range) varied systematically with latitude. Thus, a fairly consistent latitudinal environmental gradient exists along which variations in the growth and life history of soft-shell clams can be examined. Growth, modeled by the von Bertalanffy growth function, was found to be negatively correlated to latitude with temperature being the determining factor in this relationship. The relationship between growth and other life history parameters was analyzed within the context of the latitudinal environmental gradient. An association was found between decreasing latitude and the following traits: faster growth, greater variation in juvenile mortality, larger egg size, larger size at maturation, lower egg density, and decreasing longevity. These relationships suggest a consistent pattern of population adaptation along the latitudinal gradient.

**ANALYSES OF UPTAKE OF PCB'S AND TRACE METALS  
BY *MYTILUS EDULIS* DEPLOYED NEAR A DREDGE  
SPOIL DISPOSAL SITE IN EASTERN LONG  
ISLAND SOUND**

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Monitoring of two dredge spoil disposal sites in eastern Long Island Sound off the Thames River and Central Long Island Sound off New Haven has been conducted since March 1977 and April 1980, respectively. Mussels from a single population suspended 1 m off the bottom deployed near the disposal and reference sites, have been monitored for the uptake of trace metals: Cd, Co, Cr, Cu, Fe, Hg, Ni, Zn, and V, and polychlorinated biphenyls (PCB's). Analyses of PCB's were limited to six stations in the eastern Long Island Sound disposal site and only continued for 15 months. Tissues from other sites, however, were frozen and archived. In addition, histopathological and parasitological studies were initiated in January 1978.

To date the results suggest the existence of a discernible seasonal cycle in the concentrations of Cd, Cu, Hg, Ni, and Zn. During the winter and spring of 1977-1978, elevated levels of these trace metals coincided with a period of heightened disposal activity and river runoff. Similar increases were observed during 1979 and 1980, when there was little or no dredging in the river.

Tissue concentrations of PCB's increased during the disposal operations (700 ng/g), but decreased after the cessation (500 ng/g). Also, seasonal changes in the PCB levels of mussels from the disposal site were associated with the volume of spoil dumped, but this relationship was not

evident for the reference populations. Analyses of the data also revealed that the changes in the PCB concentrations of both the dumpsite and reference populations were related to the rate of Thames River discharge. However, the regression functions could account for only 20 to 40% of the observed variance and most of the variance in PCB concentrations must be attributed to factors not investigated in the present study. The regression analyses also suggest that dumping played a relatively minor role in the uptake of certain trace metals and PCB's in the monitoring populations. The results obtained to date strongly suggest the need for long-term monitoring to assess properly the effects of dredging and dumping on marine and estuarine organisms.

Histopathological examinations of the mussel tissues have yet to be completed. However, preliminary observations showed that *Chytridiopsis mytilorum* Field, a microsporidean, was the cause of destruction of the ova of the host.

**USING NATURAL RADIONUCLIDES TO MEASURE SHELL  
GROWTH RATES AND AGES OF THE BIVALVES  
*ARCTICA ISLANDICA* (LINNÉ) AND  
*PANOPE GENEROSA* GOULD**

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Shells of the bivalves *Arctica islandica* (Linné) and *Panope generosa* Gould have been analyzed for natural radionuclides as part of a study to determine growth rates of marine organisms. Before analysis shell material was scraped, soaked in warm 15% H<sub>2</sub>O<sub>2</sub>, and rinsed with 0.1N HCl to remove the periostracum.

An *Arctica* having 19 to 21 growth bands collected off the coast of Long Island, NY, had been marked 2 years earlier and returned to its habitat. The two new growth bands formed since marking indicated that banding in this specimen could be annual. The quantities of unsupported Pb-210 (22-year half-life) and Ra-228 (5.7-year half-life) present in the total shell are consistent with the growth bands being annual for a constant shell mass deposition and unsupported Pb-210 and Ra-228 contents of the shell at the time of formation equal to that measured in the portion of shell formed during the last 2 years. An *Arctica* collected off Cape May, NJ, possessed 95 to 98 growth bands and at least the most recent 72 bands deposited were deficient in

Pb-210 relative to Ra-226 (1,622-year half-life). Growth of Pb-210 toward secular equilibrium with Ra-226 in progressing back from the growing margin of the shell again suggests the growth bands are annual.

Age measurements of *Panope* collected in Puget Sound, WA, were done by measuring natural radionuclides in whole shells of young specimens with 2 to 4 growth bands and sections of shells with almost all growth bands from old specimens containing 20, 40, and 120 bands. If the composite shell sections formed from shell with a known initial Pb-210/Ra-226 activity ratio and a known shell thickening rate, then the rate of shell deposition could be calculated from the total Pb-210 and Ra-226 contents of the sections. The initial Pb-210/Ra-226 activity ratio in the young shells was 2.0. Shell thickness versus cumulative growth bands curves were measured on four shells with at least 115 bands to determine the shell-thickening rate. For these shell-growth conditions, the concentrations of unsupported Pb-210 in the integrated old samples were compatible with annual banding.

The external shell layers near the umbo often contained much higher concentrations of natural radionuclides than did layers near the growing margin. These findings usually indicate secondary additions of natural radionuclides to the older shell from which the periostracum had been partially eroded. Radiometric dating of shells requires complete removal of any secondary additions of the diagnostic radionuclides. When this was done for *Arctica* and *Panope* shells, the radiometric ages were found to be compatible with growth band ages.

#### THE OCCURRENCE OF BACTERIAL PATHOGENS OF OYSTER LARVAE: A LONG ISLAND SOUND STUDY

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A monthly series of sampling cruises in Long Island Sound provided data of seasonal shifts of bacteria over shellfish beds. The sampling stations were Loran C-positioned

in New Haven, Stratford, Bridgeport, and Norwalk. In addition to bacterial samples, water was taken for pH, dissolved oxygen, temperature, and salinity determinations. Trends in the seasonality of these data are presented.

Approximately 4 (1%) of the 464 total bacterial samples taken of the surface, bottom water, and sediment were found to be pathogenic to developing larvae of the oyster *Crassostrea virginica* (Gmelin). It is significant to note that most pathogens were isolated from sediment and surface water near the shellfish beds. This paper describes the use of selective media for the enhanced recovery of potential shellfish pathogens from salt water.

#### PREDICTIVE MODEL FOR LANDINGS OF SPINY LOBSTERS (*PANULIRUS ARGUS*) AT VARIOUS MINIMUM CARAPACE LENGTHS IN SOUTHERN FLORIDA

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Landings of spiny lobsters (*Panulirus argus*) during the 8-month fishing season were simulated through the use of a bioeconomic model. Present regulation of the fishery is conducted by the state of Florida which imposes a minimum harvest size of more than 7.6 cm carapace length. The model, based on a modified yield-per-recruit approach, evaluated the management options under consideration which included no action or implementation of a fishery management plan developed by the Gulf of Mexico and the South Atlantic Fishery Management councils. Based on selective criteria, the preferred option was implementation of a fishery management plan, using the optimum carapace length of more than 7.6 cm, ranging in size from 7.6 to 8.9 cm.

#### PREDATION OF COMMERCIALY IMPORTANT BIVALVE SPECIES IN NEW JERSEY BY THE HORSESHOE CRAB *LIMULUS POLYPHEMUS* (LINNAEUS)

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The feeding on Delaware Bay bivalve species by adult horseshoe crabs (*Limulus polyphemus* [Linnaeus]) was examined from 1977 to 1980. Both male and female horseshoe crabs fed on a wide variety of prey during the breeding season, which lasts from May until July. The most abundant bivalve on the Cape May, NJ, intertidal flats during that

time was the small venerid *Gemma gemma*, which frequently comprised over 90% of all macrofauna. However, data from stomach contents and aquarium experiments indicated that *Gemma* were consumed rarely when larger, thinner-shelled species, such as *Mya arenaria* and *Mulinia lateralis* were available. Predator exclosures confirmed the destructive impact of *Limulus* on soft-shell clam populations. In 1978, a large spatfall of *Mya* was nearly wiped out in unprotected plots; inside exclosures, *Mya* grew rapidly and reached densities of over 400/m<sup>2</sup>. A comparison of size-frequency distributions of protected and unprotected sites showed that the effect of predation was most pronounced on clams larger than 4 mm. The introduction of single adult *Limulus* into an 2.4-m<sup>2</sup> exclosure reduced the *Mya* population to ambient levels within 2 weeks.

Hard clams (*Mercenaria mercenaria*) were offered as prey in aquaria. Seed clams < 15 mm were consumed, but larger animals were resistant to crushing. When quahogs and *Mulinia* of equal number and size were offered to *Limulus*, a significant preference for *Mulinia* was evident.

In coastal waters off New Jersey, horseshoe crabs are abundant within 3 miles of the shore, from Cape May to Atlantic City, but much less common farther north. Surf clams (*Spisula solidissima*) are the most frequently occurring item in gut samples. It is speculated that the large concentration of *Limulus* in southern New Jersey may be responsible for the poor recruitment of juvenile surf clams seen in that area, at least since 1976.

#### CHANGES IN RECRUITMENT RATE AND LENGTH FREQUENCY OVER TIME IN A DEVELOPING HARD CLAM FISHERY IN THE SANTEE RIVER, SOUTH CAROLINA

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A fishery for the hard clam *Mercenaria mercenaria* utilizing escalator harvesters in three South Carolina clam beds has been monitored from its inception to the present. Catch-per-unit-effort has begun to level out after high catches of a virgin fishery. Relative abundance of juvenile clams in the catches appeared to increase in years immediately following harvest of an area. Bimodal peaks in length-frequency distribution also reflected entry of young clams into the fishery following harvest of an area. Length frequency of clams sampled outside areas open to harvesting indicated an increase in size with time, whereas in another area, they indicated little or no growth.

#### THE ROLE OF MACROCRUSTACEANS IN THE MIGRATION OF THE SOUTHERN OYSTER DRILL *THAIS* *HAEMASTOMA FLORIDANA* (CONRAD) IN MISSISSIPPI WATERS

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Blue crabs (*Callinectes sapidus* Rathbun) and striped hermit crabs (*Clibanarius vittatus* [Bosc]) provide food (attached fouling organisms) and passive transport for the southern oyster drill *Thais haemastoma floridana* (Conrad) in the outer Mississippi Sound. Ninety-nine blue crabs (98 females, 1 male; carapace width,  $\bar{X}$  = 15.2 cm, range = 11.7 to 18.3; weight,  $\bar{X}$  = 149 g, range = 71 to 269) collected in shallow waters in the vicinity of Horn and Ship islands carried 203 oyster drills ( $\bar{X}$  = 2.0/crab; range = 1 to 17); 200 drills were attached to the carapace of the crabs; drill height,  $\bar{X}$  = 36.8 mm, range = 3.0 to 73.8; drill weight,  $\bar{X}$  = 8.9 g, range = 0.1 to 53.6. The carapaces of the crabs were infested with 36.1 ( $\bar{X}$ ) acorn barnacles (*Chelonibia patula* [Ranzani]), (range = 4 to 122). Two hundred twenty-six hermit crabs that inhabited six species of gastropod shells (98 *T. h. floridana*, 67 *Busycon contrarium* Conrad, 41 *Polinices duplicatus* Say, 16 *B. spiratum plagosum* [Conrad], 2 *Murex fluvescens* Sowerby, 2 *Strombus alatus* Gmelin) carried 291 drills ( $\bar{X}$  = 1.3/shell; range = 1 to 5); drill height,  $\bar{X}$  = 18.5 mm, range = 6.2 to 43.4; drill weight,  $\bar{X}$  = 1.2 g, range = 0.1 to 11.2.

The oyster drill/blue crab association persisted during the late summer and early fall as the spawning female crabs congregated in the shallow waters around the barrier islands and ceased when those crabs died or left the nearshore waters during the winter. The oyster drill/hermit crab association existed concomitantly but persisted into late fall. Drills attached to blue crabs were twice the mean size of those attached to hermit crabs (36.8 versus 18.5 mm, ht). Evidence suggests that the drills preyed on barnacles on both crabs and on small oysters (*Ostrea equestris* Say) attached to the hermit crab shells. Prey availability (fouling organisms), potential food source (postmortem crab tissues), and passive transport are advanced as possible reasons for the drill/crab associations.

Blue crabs also served as "transport" for three other gastropods: *Cantharus cancellarius* (Conrad), *Crepidula plana* Say, and *Odostomia impressa* (Say). Oyster drills were found on two other invertebrate species: *T. h. floridana* (alive) and the horseshoe crab *Limulus polyphemus* Linnaeus. Crabs "acquired" their drills while buried in the substrate, under peat outcroppings, or while resting among marsh grass roots or other debris, during which the drills

attached via their muscular foot. Once attached the drills were not easily dislodged by the movement of the "host" crab. Blue crabs with attached drills were taken incidental to the study in shrimp trawls and crab pots between the barrier islands and the Mississippi mainland.

#### ANALYSIS OF AGGREGATE FISH AND SHELLFISH EXPENDITURES

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Operations and investment planning on the part of the seafood industry requires reliable measures of consumer expenditure patterns. Consumer behavior depends upon changes in relative prices, changes in income, and changes in socioeconomic and demographic characteristics. However, a paucity of information exists as to how such changes affect the seafood industry. This study investigates the nature and magnitude of the influence of socioeconomic and demographic variates on fish and shellfish expenditures. The list of characteristics includes: (1) geographic region, (2) location of residence, (3) family size, (4) race, (5) marital status, (6) education, (7) occupation, (8) industry, (9) income, (10) season, (11) tenure class, and (12) age and sex composition. The source of data is the 1972-1974 U.S. Bureau of Labor Statistics Consumer Expenditure Survey.

#### AN EXPLORATORY STUDY WITH PROTON MICROPROBE OF THE ONTOGENETIC DISTRIBUTION OF ELEMENTS (Na TO Sr) IN SHELL OF LIVING OYSTERS

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The possible use of the shells of bivalves as indicators of trace metals in the environment poses serious problems because of unknown biological variables. A major problem is lack of adequate knowledge of how chemical composition of shell changes during the life history of each individual. We report the results of a preliminary experiment designed

to determine the feasibility of monthly monitoring, with the proton microprobe, the chemical elements in the mid-ventral margin of the right valve of three experimental and six control groups of living, young, attached oysters (*Crassostrea virginica* [Gmelin]) cultured (fed algae, seawater changed ever other day) in controlled laboratory conditions for 3 months. The study, possible because use of the probe does not require sacrifice of the animal, had not been attempted before. Potential danger from irradiation was obviated by analysis of the ventral margin of the shell from which mantles retract when valves close. The probe scanned a rectangle  $2.5 \times 0.5 \mu\text{m}$  and analyzed the spectrum of 16 elements from Na to Sr in concentrations as low as a few ppm. A microprocessor stored the data. Quantitation was done by standardization against curves obtained with the probe of known concentrations of elements normally found in shell mixed in pure  $\text{CaCO}_3$ . Elements are reported as percent concentrations by weight (of elements analyzed, not of shell).

All but two control oysters survived the 4 monthly analyses and increased in mean weight from 0.23 to 4.0 g during the 3-month monitoring period. Although considerable fluctuation in concentration of different elements occurred among different individuals and at different stages, variations for any one element generally were nominal. Overall, S, Mn, Fe, and Zn exhibited a light increase in concentration (possibly from algal nutrients) in successive ontogenetic stages, whereas Na, Mg, Al, Si, Cl, K, Ca, Ti, Cr, Cu, Br, and Sr remained relatively constant when present. This relative constancy is what might be expected of oysters raised in relatively uniform environmental conditions. These results indicate initial promise for the approach and suggest longer-range studies in different types of experimental environments.

#### THE EGG CAPSULE OF *ARCTICA ISLANDICA* (LINNÉ)

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Ova were collected from adults of the ocean quahog *Arctica islandica* (Linné) by stripping and from spawnings induced by temperature shock. Spawners were subjected to

water temperature changes from 14 to 29°C. The eggs were treated with a 3% solution 0.1N  $\text{NH}_4\text{OH}$  for 15 to 20 minutes at 15°C and then allowed to develop and grow at 12 to 15°C. Only a few of the eggs from the induced spawning developed to straight hinge. Several thousand straight-hinge larvae were obtained from the stripped eggs.

The eggs were interesting in that each ovum was encased in a capsule with an apical pore. The capsule walls appeared to be relatively thick and impervious. Sperm was not observed to penetrate the capsule and it was assumed that sperm entered through the pore.

Sometimes the eggs started development within the capsule and occasionally were observed leaving the capsule by squeezing through the pore. These eggs usually failed to develop beyond the 4-cell stage.

More commonly the capsule appeared to dissolve and rupture after fertilization. Often the ruptured capsule appeared as a tuft-like appendage at one of the poles of the egg during early cleavage stages.

The implication of encapsulated eggs is interesting. This species inhabits relatively deep, cold, high-salinity water with little temperature or salinity fluctuations. An encapsulated egg may be thought of as useful in a more changeable environment but seems somewhat superfluous to *Arctica*.

#### LARVAL AND POSTLARVAL DEVELOPMENT OF THE BURROWING CLAM *DIPLOTHYRA SMITHII* TRYON

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*Diplothyra smithii* Tryon is a small bivalve mollusk that burrows into the calcareous shell material of the American oyster *Crassostrea virginica* (Gmelin). Larvae of the burrowing clam were reared to the juvenile stage at the Eastern Shore Laboratory of the Virginia Institute of Marine Science, Wachapreague, VA, and postlarvae were reared at the Gulf Coast Research Laboratory in Ocean Springs, MS.

Clams spawned in the laboratory at water temperatures between 23.7 to 29.5°C. Larvae were reared in water salinity at 30 ppt and temperatures between 16 and 24°C. Straight-hinge veliger larvae were noted 24 hours after fertilization, and for up to 7 days after fertilization. The umbo stage was noted first 4 days after fertilization, and the pediveliger stage on day-24. Larval dimensions of *D. smithii* were

smaller than those reported for related pholads; however, the hinge structure and the shape of the shell were similar to those reported for other pholads. Metamorphosis began on day-29 and occurred only with the addition of shell substrata. The length of the larval period correlated well with subsequent laboratory and field studies on spawning and setting times for *D. smithii* in Mississippi Sound.

Postmetamorphic clams were reared until day-61. Serrate shell margins were observed first on day-39. This shell growth feature, present in the anterior-ventral region, was important for both initial and sustained mechanical penetration of calcareous shell material.

#### FACTORS OF MORTALITY AND GROWTH IN AN INTER-TIDAL POPULATION OF JUVENILES OF *MERCENARIA MERCENARIA* FROM SHARK RIVER, NEW JERSEY, OVER A TWO-YEAR PERIOD

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The mortality of a population of lower-intertidal juveniles of *Mercenaria mercenaria* was monitored from September 1979 through July 1981 in the Shark River estuary in New Jersey. Over 800 juveniles/m<sup>2</sup> were present at the beginning of the study; however, more than 75% of these clams died during the first 6 months. These losses were due almost entirely to predation by brachyurans and ducks, and predation was most severe on individuals over 7 mm in length. A negative power function ( $N_t = N_0^* t - 2.072$ ) was fitted to the mortality data, with current juvenile density being 23 individuals/m<sup>2</sup> (March 1981). The mean length of juveniles when first quantified was 5.3 mm in October 1979; by March 1981, the mean length had increased to 13.6 mm. Disease appeared to have affected clams in the 2- to 3-mm size range, particularly during the spring. Based on these studies, a life table projection was constructed which showed that the life expectancy of juveniles was very low through the first 2 years of life, but increased rapidly after 3 years. The life table data also predicted an equilibrium population level of 30 adult clams/m<sup>2</sup>. The absence of certain year classes in adult populations of clams probably was due to set failures in the past.

# A ONE-YEAR DISEASE SURVEY OF FOUR COMMERCIALY IMPORTANT, RHODE ISLAND BIVALVE MOLLUSKS:

*MYA ARENARIA*, *ARGOPECTEN IRRADIANS*  
*IRRADIANS*, *CRASSOSTREA VIRGINICA*,  
AND *MERCENARIA MERCENARIA*

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The prevalence of parasitism in four commercially important mollusks was determined from feral populations collected seasonally from Rhode Island waters. The selection of the four species was based on their economic importance, and on their potential use as mariculture species. Sampling was conducted from June 1979 to May 1980. All four species harbored parasites that could affect their ability to be grown under mariculture conditions. Because of the high prevalence of the hematopoietic neoplasm (37%) in the soft-shell clam *Mya arenaria* from Rhode Island waters, it is suggested that mariculture stocks be brought in from Chesapeake Bay. The bay scallop *Argopecten irradians irradians* was parasitized heavily by trematodes, rickettsiae-like organisms, and coccidians, that could affect the success of culture operations. The American oyster *Crassostrea virginica* was parasitized only lightly, and no evidence of "Dermocystidium" or "MSX" was observed. The hard-shell clam *Mercenaria mercenaria* was the least parasitized of the four species examined. Based on these observations, the American oyster and the hard-shell clam populations were the healthiest, and might be considered as sources of stock for mariculture operations.

# SEASONAL GONADAL DEVELOPMENT OF YOUNG LABORATORY SPAWNED NORTHERN (*MERCENARIA MERCENARIA*) AND SOUTHERN (*MERCENARIA CAMPECHIENSIS*) QUAHOG CLAMS AND THEIR RECIPROCAL HYBRIDS IN NORTHWESTERN FLORIDA

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Beginning in November 1974, and continuing at monthly intervals for 1 year, northern and southern quahog clams and their reciprocal hybrids, that had been spawned the previous April, were examined histologically for gonadal

condition. All four groups developed as males (0.3% initially as females). The northern species had ripe follicles throughout the period (except the first, November 1974), and there were spawnings with peaks in February and April 1975, and with minor spawnings throughout the summer. No ripe follicles were seen in the southern quahog during the summer, May-August (2% in July), as well as the first examination in November 1974. Peaks of spawning occurred in December 1974 and February 1975, with lesser spawning indications through April. The two hybrids followed the cycle of the northern quahog parent more closely than the southern.

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# SEASONALITY AND DISCRIMINATION IN THE FEEDING BEHAVIOR OF THE SOFT-SHELL CLAM *MYA ARENARIA* LINNÉ

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Filtration rates, food intake, and absorption efficiencies of *Mya arenaria* Linné were investigated on a seasonal basis (spring, 3 to 8°C; summer, 14 to 20°C; autumn, 3 to 8°C) in a flow-through seawater system. In each season, a range of concentration of two types of particulate material were fed as follows: (1) resuspended mudflat sediments (2 to 3% carbon by dry weight), and (2) laboratory cultures of the diatom *Thalassiosira pseudonana* (9 to 12% carbon by dry weight). The relationships of filtration rate and amount of food cleared from suspension to ambient particulate concentrations showed different seasonal patterns for the two particulate types. In the summer, spawned-out clams were able to process and ingest larger numbers of diatom cells, and to digest them slightly more efficiently than in the spring. In the autumn, the amount of algae processed again decreased but remained well above spring levels. Resuspended mudflat sediments were cleared from suspension most rapidly in the spring, and carbon and protein measurements of the biodeposits suggested that significant nutritive inputs were obtained from the diet. Feeding on resuspended mudflat sediments was enhanced by the addition of small amounts of *Thalassiosira pseudonana*, indicating that *Mya arenaria* is able to discriminate between diets of differing quality. Results of particle-sorting experiments indicate that *Mya* is able to partially sort mineral particles from organic particles prior to ingestion.

**SHELL FRAGILITY, GROWTH DEPRESSION, AND MORTALITY  
OF JUVENILE AMERICAN AND EUROPEAN OYSTERS  
(*CRASSOSTREA VIRGINICA* AND *OSTREA EDULIS*)  
AND OF HARD CLAMS (*MERCENARIA MERCEN-  
ARIA*) ASSOCIATED WITH SURFACE  
COATING *VIBRIO* SPP. BACTERIA**

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Growth depression, shell abnormalities, and mortalities of cultured juvenile American and European oysters (*Crassostrea virginica* and *Ostrea edulis*, respectively), and mortalities of hard clams (*Mercenaria mercenaria*) were reported from three hatcheries and nursery facilities in Massachusetts and Maine in July 1980. Affected oysters were examined grossly, microscopically with scanning electron microscopy, and histologically. Bacteriological samples were taken from the culture system container surfaces, from the water column at various points in the system, and from oyster- and clam-shell surfaces.

Juvenile oysters from all groups were from 2 to 12 mm in shell height; oysters from all groups showed peripheral shell fragility, lack of calcium salt deposition, large areas of chalky deposits, and overgrowth of the left valve. Attached bacterial cells were associated with erosion of the peripheral periostracum. Histological examination revealed the erosion of the ligament associated with a bacterial infection, and also suggested a low rate of normal metabolic activity in digestive gland absorptive cells.

Nearly pure, uniform cultures of clear-spreading bacteria predominated the isolations from all clam- and oyster-shell surfaces and from all container surfaces, but were not detected in any water column samples. Biochemical characterization of the bacteria, including determination of DNA-base ratios, demonstrated that the shell surface bacteria were two similar strains of *Vibrio* spp. (one from clam-shell surfaces in Massachusetts, and the other from oyster-shell surfaces in Maine). The moles percent guanine plus cytosine for the isolates varied from 43.6 to 44.5%. These microbes showed markedly increased growth rates from > 25° to 30°C. Antibody was produced in chickens to the isolates; specificity testing demonstrated that the antibody methods were the most precise and rapid means of identifying and differentiating the closely related marine bacteria.

The temperature response and acid-producing characteristics of the isolates appear to be related to their effects on juvenile shellfish. The condition can be managed easily in culture systems by upgrading water treatment and hygiene procedures. Practical monitoring programs for early detection of the condition are described.

**PATHOGENESIS AND MICROBIOLOGICAL CHARACTERIZA-  
TION OF A BACTERIAL INFECTION OF CULTURED  
JUVENILES OF THE RED ABALONE  
*HALIOTIS RUFESCENS* SWAINSON**

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This report summarizes the pathogenesis and bacteriology of an infection of juvenile red abalone (*Haliotis rufescens* Swainson). Although recent efforts have led to the successful development of an intensive system for culture of red abalone, occasional high rates of mortality can impair production of the animals. Dying abalone were removed from culture tanks over a 9-day period in August 1980 to investigate mortality phenomena. In addition, 48 animals (7 mm to 14 mm in shell length) were subjected to supersaturated oxygen conditions, and approximately 2,000 animals were subjected to an elevated temperature to make a preliminary evaluation of the effects of these stresses on the abalone.

All moribund animals selected for sampling were examined grossly, microscopically, and histologically. Foot-muscle tissue was sampled for bacteriological evaluation. Surface and water column samples from culture tanks also were taken for bacteriology. Seven isolates of over 20 initial isolates from both animal tissue and culture tanks were selected for detailed biochemical characterization, and for antibody production.

Clinical observation of affected animals from culture tanks, and stress experiments showed the apparently non-specific findings of loss of epipodial, foot, and mouth pigmentation, retraction of the cephalic tentacle, and the inability of the animal to maintain attachment with the substrate. Histological observations of moribund animals from all sources demonstrated bacteria attached to soft tissue surfaces and a characteristic mode of infection. Following exfoliation of foot, mantle, or epipodial epithelium, the bacteria demonstrated an affinity for vascular and neural endothelium. Thus, the bacteria rapidly infected all

anatomical areas by preferential growth along the linings of vascular spaces and neural structure endothelium. The specific fluorescent antibody test used on frozen tissue sections demonstrated that the described pathogenetic pattern was associated with a particular bacterial isolate, designated "GA." Further observations indicated that bacterial infection of digestive tract epithelium and gill tissue occurred less frequently.

Detailed biochemical characterization suggested that all of the seven isolates conform to the currently accepted definition of the genus *Vibrio*. Although several isolates were similar biochemically, all isolates could be distinguished rapidly using the antibody methods.

Isolates such as GA were made from all sources, but were isolated most consistently from tissues of animals subjected to supersaturated oxygen stress. The bacterial infection observed here apparently resulted from the effects of a highly opportunistic bacterium. Further, it seems likely that physico-chemical or other stresses, in addition to the presence of bacteria, are required for infection to occur. Thus, management of the condition may depend both on reducing numbers of opportunistic bacteria, and on eliminating occasional stresses that occur during the culture process.

#### SHELL GROWTH OF THE HARD CLAM *MERCENARIA MERCENARIA* (L.)

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Hard clams (*Mercenaria mercenaria* [L.]) (n = 315) approximately 13 months old were individually marked and planted in a subtidal location in Clark Sound, SC. Shell dimensions (length, height, width) and growth (change in shell size) of individual clams were monitored over 4.5 years. Most growth occurred in the first 2 years. Correlation coefficients computed between initial shell sizes (at planting) and growth were negative, which suggests compensatory growth. In contrast, all relationships between initial shell sizes and shell sizes after 4.5 years growth were positive.

These data indicate that (1) smaller and larger clams in the population remained within the same segments of the size distribution throughout the experiment, and (2) smaller clams were growing faster than larger clams. Intuitively, variances of the size distribution are expected to decrease over time; however, they remained constant. These results are discussed in relation to the mechanisms of growth adjustments in molluscs.

#### ANNULUS FORMATION IN MICROSTRUCTURE OF THE HARD CLAM *MERCENARIA MERCENARIA* (L.) IN VIRGINIA

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Hard clams (*Mercenaria mercenaria* [L.]) from a 12-year growth study were examined in radial section using acetate peels to identify annual growth patterns, distinguish the time of year of their formation, and describe growth throughout the year using daily increments. The disturbance to the clam caused by annual fall measurements left a characteristic growth interruption mark in shell microstructure. This mark was consistently preceded in shell microstructure by an extended period (40 to 200 daily increments) of slow growth in the summer of each year. This appeared in macroscopic and microscopic views of shell cross sections as a dark band across the middle homogenous layer. The number of dark bands in clams that were not measured in a series of years was equal to the number of years (summers) in the period. An extended period of slow growth has been reported to be formed each winter in northern populations of hard clams. In Virginia, a homologous slow growth period is formed each summer.

Another group of 3-year-old clams were planted in subtidal bottoms in the York River and were sampled monthly for 1.5 years. The dark band of slow growth appeared first in July, from examination of the shell-edge growth patterns. Growth was interrupted frequently in many clams throughout the summer, presumably as a result of high-water temperatures. As temperatures declined in the fall, the growth rate increased producing a lighter band of shell growth which first appeared in September. This lighter band of shell growth continued to be formed throughout the winter, despite growth interruptions by cold-water temperatures in February. No extended period of slow growth producing a dark band has been observed in hard clams sampled in winter or spring from this group. As water temperatures rise in the spring, growth interruptions become less frequent and growth rate increases.

## SEASONAL PATTERN OF CONDITION INDEX OF MUSSELS IN THE LAGUNA VENETA

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Seasonal changes in condition index (CI) and lipid content of mussels (*Mytilus edulis*) were followed by monthly sampling from 1975 to 1980 in two populations in the southern basin of the Laguna Veneta (NE Italia). The CI (ratio of soft tissue weight to shell cavity volume) was determined on the basis of both dry and cooked meat weights. Extractable lipoids also were determined in the dried tissues. A strict correlation was demonstrated between dry weight and cooked weight CI values. The CI varied as much as three-fold from a winter low to a summer high. The period of high CI appears to correlate with greater phytoplankton abundance. Mussels of one population, residing in waters heavily polluted with urban wastes, tended to maintain a better condition during winter months when phytoplankton levels were very low (chlorophyll-a < 1.0 µg/l). The pattern of lipid content (< 2% to > 12% of dry tissue weight) was more complex and probably reflected the interaction of food availability and gamete production and emission.

## CAN PHYTOPLANKTON CARBON/NITROGEN RATIOS BE OF NUTRITIONAL SIGNIFICANCE FOR BIVALVES?

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The growth of juvenile Manila clams (*Tapes philippinarum*) was examined on diets of the diatom *Thalassiosira pseudonana* (3H) of similar cell concentrations but of differing carbon/nitrogen (C/N) ratios (by atoms) over a period of 4 weeks. Bivalve tissue growth was correlated with the total quantity of dietary nitrogen available rather than dietary carbon; however, dietary C/N ratios of 8.4:10.5 appear superior to higher or lower values. The use of C/N ratios as an indicator of nutritional value in aquaculture food species, and measurement and interpretation of the ratio are critically reviewed.

## COMMERCIAL FISHING OPERATIONS OFF SOUTHERN NEW ENGLAND FOR THE DEEP-SEA RED CRAB

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Data collected from two sampling trips aboard a commercial red crab vessel were used to document red crab fishing operations, to determine a section weight/live weight relationship, and to obtain size-frequency measurements and sex ratio of the commercial red crab catch and discard. Red crab fishing took place on the upper continental slope in the Hudson Canyon area in July 1979, and in the Atlantis-Veatch canyons area in October 1979. Trawls, or strings of wooden offshore lobster pots attached to a groundline, were fished for the deep-sea red crab. Adult male crabs (generally  $\geq 114$  mm in carapace width) were butchered at sea; a conversion factor 1.71 adjusts section, or landed, weight to live weight. Commercial size male crabs ranged from 93 to 150 mm in carapace width on both trips; small, discard males ranged from 77 to 137 mm; and discard females ranged from 80 to 126 mm. A significant difference from a 1:1 sex ratio did occur in discard from the July trip; however, size differences between the two trips were minimal.

ENZYME PATTERNS IN THE SEA SCALLOP *PLACOPECTEN MAGELLANICUS* (GMELIN) WITH RESPECT TO ENVIRONMENTAL VARIABLES, ANIMAL SIZE, AND CADMIUM EXPOSURE

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Biochemical examination of the phasic adductor muscle of sea scallops (*Placopecten magellanicus* [Gmelin]) recently collected from waters off the mid-Atlantic coast, and from deep-water stations in the Gulf of Maine showed different patterns of glutamate dehydrogenase (GDH) activity. In animals from the more southerly stations, GDH seemed to be related to shell size, with much higher activity in the smaller adults (8 to 10 cm) than in the larger ones (11 to 15 cm). This relationship was not observed in the deep-water scallops (155 m); adductor muscle GDH in these nutritionally deficient animals, however, was twice ( $< 0.001$ ) that found in scallops from a nearby, shallower station

(68 m). The deep-water animals also had very low muscle glycogen and low pyruvate kinase activity.

Experimental exposure of sea scallops, average size 10 cm, to 10 ppb Cd for 30 and for 60 days while undergoing heat stress (from 15°C at 30 days to 18.7°C at 60 days) produced the following observations on adductor muscle biochemistry: increased GDH ( $< 0.001$ ) in both control and exposed animals taken from 18°C water, with no metal effect at either temperature; lowered citric acid cycle activity (IDH,  $< 0.01$ ) in controls at 18°C, with the effect abolished ( $< 0.002$ ) in Cd-exposed animals; increased malate dehydrogenase ( $< 0.05$ ) in controls at 18°C, with the effect abolished ( $< 0.001$ ) in the Cd-exposed animals; and increased aspartate aminotransferase ( $< 0.005$ ) only in the Cd-exposed animals at 18°C (synergism). Because apparently normal hormetic responses (for IDH and MDH) in scallops under heat stress were not seen in the Cd-exposed scallops, this study provides one more example of Cd-induced attenuation of an adaptive physiological mechanism.

#### POPULATION STUDIES OF THE PACIFIC OCTOPUS *OCTOPUS DOFLEINI* (WULKER)

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Studies are being carried out on octopuses inhabiting shallow waters on the western coast of Vancouver Island, British Columbia. Denning behavior has been observed and a den-octopus size relationship established. A removal experiment indicated rapid recruitment at certain times of the year. Current tag-and-recapture studies are providing growth and survivorship information and mobility data.

To date, these studies indicate that *Octopus dofleini* (Wulker) is an abundant inhabitant of the west coast, capable of rapid growth, subject to high risks from predation and cannibalism, and worthy of consideration in terms of fisheries and mariculture potential. Sex ratios suggest differential behavior or mortality in males and females. Onshore-offshore migrations may be occurring.

#### ENVIRONMENTAL INFLUENCES ON MSX INFECTION PATTERNS IN DELAWARE BAY

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Epizootic mortalities of oysters in Delaware and Chesapeake bays during the late 1950's and early 1960's diminished in an upbay direction, indicating that the causative agent, the sporozoan parasite *Haplosporidium nelsoni* (formerly *Minchinia nelsoni*) (MSX), was salinity-limited. Since 1959, native Delaware Bay oysters have been sampled for presence of MSX and for mortality along a salinity gradient that ranges at mid-tide and mean river flow from 23 to 20 ppt on the lower bay leased grounds, and from 18 to 9 ppt on the upper bay seed beds. This study has clarified the relationship of salinity to the distribution and effect of the parasite on native oysters in the estuary.

The sampling period included a drought in the mid-1960's when very low Delaware River flows produced high salinities in the upper bay, as well as a period of very high river flows, and low salinities, in the early 1970's. On Arnolds and Cohansey, two of the uppermost seed beds, with mean mid-tide salinities of 9 and 12 ppt, respectively, MSX activity was significant only during the lowest river flow periods at the peak of the drought. Farther down-bay, at New Beds and Bennies Bed (15-16 ppt), the disease was present in all years but with clearly enhanced activity during the drought. On the leased grounds, however, where the effects of MSX are felt most strongly by the oyster industry, there was no correlation of disease levels with river flow. In fact, MSX levels were higher in the early 1970's when river flows were high than during the drought in the mid-1960's.

When disease and mortality statistics for each location along the salinity gradient were pooled for the entire 20-year sampling period, two distinct patterns emerged.

1. Prevalence (percentage of oysters diagnosed as having MSX) of disease showed a regular decrease from high-to-low salinity that paralleled the salinity gradient.

2. Infection intensity, a better measure of disease stress than prevalence, however, showed a sharp drop between planting grounds and seed beds, then no further change with lower salinity.

MSX-related mortality followed a pattern similar to infection intensity: on a long-term average, 30% of all oysters have died with MSX infections during their first year after planting on the leased grounds. On the seed beds, regardless of locations, annual disease-related kill was only 4 to 9%.

The sharp drop in infection intensity and in MSX-related mortality occurs between the upbay edge of the leased grounds and the lowermost seed bed, locations that are separated by less than 3.2 km and a salinity mean of only 2 ppt. This suggests a salinity threshold which has little effect on the distribution of infective stages of the parasite or on its ability to infect, but severely limits the capacity of the parasite to develop once it has entered the oyster.

# GROWTH OF CYTOCHALASIN-INDUCED TRIPLOIDS OF THE AMERICAN OYSTER *CRASSOSTREA* *VIRGINICA* (Gmelin)

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Triploid oysters were produced by treating fertilized eggs with cytochalasin-B (CB) (Sigma Chemical Corp., St. Louis, MO). Eggs treated at meiosis I (0.5 mg/l CB in 0.005% DMSO) at 0 to 15 minutes post-fertilization produced 60% triploid oysters whereas those treated at meiosis II (15 to 30 minutes post-fertilization) were 73% triploid as determined by the flow cytometry and chromosome counts. The remaining oysters within the groups were diploid, and served as true controls in subsequent growth experiments because they were reared in consort with their triploid siblings from fertilization onward.

Field experiments, initiated in the third year of life, compared linear growth of triploid oysters with diploid siblings, with a final sacrifice of some animals to compare dried meat weights. At all five measurement periods, the meiosis I triploids were significantly larger ( $P \leq 0.001$ ) in mean length than their diploid controls, whereas triploids created at meiosis II were not significantly different than their diploid controls. The sacrificed samples (19 meiosis I triploids and 18 within group diploids) revealed dried meat weights for triploids at 0.43 g versus 0.31 g for diploids, a difference that was significant at the 0.05 level.

Preliminary scans of histological sections revealed that gametogenesis may not be blocked by triploidy; therefore, the mechanism of growth enhancement may be through increased heterozygosity with triploidy. These mechanisms are under investigation and will be reported more fully in the future.

# THE EFFECTS OF ANTHROPOGENIC ENVIRONMENTAL CHANGE ON REPRODUCTION AND CONSEQUENT POPULATION STRUCTURE OF THREE SYMPATRIC TEREDINID SHIPWORMS

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The Oyster Creek Nuclear Generating Station on Barneget Bay has caused increased temperature, salinity, and turbidity in Oyster Creek and in parts of Forked River, NJ. The thermal effluent is absent during the frequent station outages. Two native species of the marine woodboring

bivalve family Teredinidae, *Banka gouldi* and *Teredo navalis*, and the subtropical introduced species, *T. bartschi*, have been studied in Oyster Creek to determine patterns of reproduction and population structure as related to the environmental changes. The species have planktotrophic, short-term larviparous, and long-term larviparous larvae, respectively. The increase in salinity has allowed all three species to reproduce in creek areas that formerly were able to support breeding populations only during years of drought. Because temperature is related to growth rate, and size is related to reproductive output, the thermal effluent has increased reproduction in all species. The increase in temperature has extended the breeding season of *T. navalis*, but has had less of an effect on *B. gouldi*. *Teredo bartschi* has the longest breeding season. Its larvae are stored in the gills over winter, allowing the species to respond rapidly to favorable environmental conditions. *Teredo bartschi* has a very high percentage of functional females and may be a simultaneous rather than a protandrous hermaphrodite. Self-fertilization could be a possibility. The implications of the brooded larval development and other life history characteristics of the introduced species are that it is better able to track the fluctuations of the environment than native species, but its population is patchy in time and space. The findings are relevant to other marine bivalves in thermal effluents.

# USE OF GROUP TESTING THEORY IN MARINE RESEARCH

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Group testing is a technique for estimating parameters of a statistical population using observations on groups rather than on individuals. For example, if one is interested in the proportion of mussels with an intestinal copepod, one can randomly assign mussels to jars (groups), add a digestive enzyme and examine the residue for parasites. Then the proportion of groups exhibiting the trait (parasite) is related to the proportion of individuals in the population with the trait.

The maximum likelihood estimator for the binomial proportion has been shown to have a positive bias. Methods of reducing this bias will be presented and the design of experiments will be discussed in terms of controlling the precision of the estimator.

To effectively use group testing it must be easier to test several individuals simultaneously than it would be to test them separately. The incidence of the trait(s) of interest must be sufficiently small to assure a saving of effort. Group testing can make prohibitively large projects feasible.

Some possible applications to shellfisheries research are the detection of pathogens in culture, the staining of spores in macerated tissues, and the detection of various substances indicative of disease or genetic or racial composition by biochemical test or electrophoresis.

#### FEEDING, FATTENING, AND GROWTH OF THE AMERICAN OYSTER AT ELEVATED TEMPERATURES

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The annual cycle of condition of the American oyster is well known. Historically, fishermen and biologists consider the poor quality of oysters during summer and early fall to be an incompatibility of oyster physiology with high water temperatures, and the supposedly debilitating effects of spawning.

Through the years, experimenters have provided some evidence that oysters are not irretrievably evolved to be of low glycogen content in warmer months. Although watery flesh and poor quality appear to be ubiquitous during the summer, various workers have suggested that glycogen could be increased and quality restored simply by making suitable food available in abundance.

We present the results of two experiments using large numbers of oysters (40 and 60 bushels, respectively) and powdered cornmeal as food. Water temperatures averaged 29.8 to 31.0°C in the first experiment. Ranges were 27.6 to 34.2°C in the second. In the latter experiment, temperatures reached or exceeded 34.0°C on 4 of 21 days of the observations.

Not only did oysters show large increases in glycogen content, but also exhibited rapid new growth in the second flow-through experiment. For example, one oyster showed a new shell production of 1.27 cm in 19 days. After 2 weeks of feeding, mortality began to reach unacceptable levels in the first experiment.

The cause of these mortalities was identified (insufficient water exchange). Partial correction of the inadequacy was made in the second experiment with reduced mortality.

As might be expected, fairly rapid flow-through appeared to be essential when oysters were fed in warm water.

#### GROWTH HISTORY OF *SPISULA SOLIDISSIMA* DILLWYN AS REVEALED BY OXYGEN ISOTOPES AND SCLEROCHRONOLOGY

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Stable isotopic and sclerochronologic studies were performed on surf clams (*Spisula solidissima*) collected live from above and below the seasonal thermocline on the continental shelf off New Jersey. Isotopic analyses were made across ten entire annual shell-growth increments deposited during 1965-1976 in an inshore (10-m depth) specimen and across the calendar year 1966 in two offshore (45-m depth) specimens.

Profiles of  $\delta^{18}\text{O}$  across annual shell-growth increments in clams from above and below the thermocline track the monthly mean temperature trends in shallow and deeper shelf waters, respectively, providing further strong evidence that these growth increments indeed are annual. Size of annual growth increments throughout ontogeny predicted by sclerochronology are confirmed by the annual cyclicity of  $\delta^{18}\text{O}$  values. The annual isotopic amplitudes ( $\Delta^{18}\text{O}$ ) are greatly reduced during years which were less conducive to shell growth (warmer than average). A linear relationship exists, however, between  $\Delta^{18}\text{O}$  values and annual mean sea surface trends.

Records of  $\delta^{13}\text{C}$  reflect the seasonal cycling of nutrients within the water column. Values of  $\delta^{13}\text{C}$  for clams living in shallow and deeper waters are similar when shelf waters are well mixed during winter months, but diverge sharply following thermocline development. This divergence reflects the transfer and storage of  $^{12}\text{C}$  to shelf waters below the thermocline during the summer months. The  $\delta^{13}\text{C}$  values then return abruptly to original values upon fall mixing of the water column.

The surf clam faithfully records much of the oceanographic-climatic variability of the inner continental shelf in the structural and chemical variations within its shell.

# **THE DEVELOPMENT OF A RACEWAY CULTURE AND FIELD GROWOUT PROGRAM FOR *MERCENARIA MERCENARIA* IN THE TOWN OF BROOKHAVEN**

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The shellfishery for hard clams (*Mercenaria mercenaria*) in the Town of Brookhaven has a dockside value of over \$5 million annually. Because of a decline in landings from 220,000 bushels in 1978 to 109,000 bushels in 1980, the Town has initiated a mariculture program to provide juvenile clams (~ 25 mm in length) for restocking of the public bay bottom. For this type of clam reseeding to be an effective management tool, it must be able to produce large numbers of clams at a relatively low cost.

To meet that requirement, the Town of Brookhaven has developed a 2-step growout procedure utilizing onland raceways and field growout. Unlike other procedures (rafting and bottom culture), which require starting with 4-mm seed, this approach enables use of the cheaper and more readily available 0.5-mm postset. The postset are purchased from hatcheries and grown in the raceway until they attain 4 mm. At that size, they are transferred to the nursery growout system, based on methods developed by Castagna, where they are kept until reaching 25 mm. At 25 mm, they are harvested and planted into the shellfish beds. The process takes 18 months and survival through the two steps is approximately 50%.

Raceway-field growout has several advantages: (1) the raceways enable use of 0.5-mm seed, (2) the technology for both phases is simple, (3) costs are reduced relative to other methods, and (4) the procedure can produce the volume of seed needed for restocking. I believe that by using this system, mariculture can be used to effectively and efficiently augment natural production.

# **GAMETOGENIC CYCLE OF THE HARD CLAM *MERCENARIA MERCENARIA* FROM DIFFERENT LOCATIONS IN GREAT SOUTH BAY, LONG ISLAND, NEW YORK, AND ITS MANAGEMENT IMPLICATIONS**

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Changes in the reproductive condition of the gonad of the hard clam *Mercenaria mercenaria* at five locations in Great South Bay, Long Island, NY, were followed for

over 2 years. From microscopic evaluation of histologic sections, the gametogenic cycle was described by using qualitative criteria to assign each animal to one of five developmental stages. To facilitate analysis, each stage was given a numerical value which permitted the calculation of a gametogenic index for each sample.

A complete gametogenic cycle was found to occur only once annually. Hard clams were mature by the end of May, and spawning occurred between mid-June and early July. Production of oocytes for the next spawning began in the fall. Comparison of the gametogenic index over time and location showed that annual differences were more apparent than location differences. In 1978, the spawning period was short and well defined, while in 1979, spawning occurred over a longer period of time. A comparison of the gametogenic cycle among clams of three size classes revealed no significant size-specific differences. These observations can be used in the development of a management plan for the species.

# **ENVIRONMENTAL INFLUENCES ON SEX RATIOS AND SPAWNING IN OYSTERS**

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Two aspects of reproduction in *Crassostrea virginica* require further study. One centers on the effects of different environmental influences on sex ratios in nature, and the other involves possible variations in spawning stimuli throughout the range of the eastern oyster.

Most young of *C. virginica* are functional males. As they age, many become functional females. However, on oyster grounds, with limited recruitment of young (male) oysters in Chesapeake Bay, ratios of females to males generally are less than 2:1, although greater than 1:1. How do oysters compensate for paucity of young males in the population? Limited evidence in the literature indicates that proximity of other oysters may influence sex. In addition, it is possible that stressful conditions lead to a more balanced (1:1) sex ratio.

Temperature plays an important role in spawning. However, is spawning triggered by a temperature rise to a certain level, by a rapid rise after ripening, by the presence of a specific food material, or by some combination of these or other factors? It appears that a combination of factors may be involved, at least in some (more southerly?) locations.

## PRICE FLEXIBILITY ANALYSIS OF VIRGINIA HARD CLAMS

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The study of ex-vessel price flexibility of Virginia hard clams (*Mercenaria mercenaria*) was conducted using historical analysis of monthly landings and average prices over the period 1960-1979. Results depict relative impact on average prices received by the harvesters due to fluctuations in hard clam catches. Various seasonal price-quantity relationships were also examined to investigate changes in price flexibility resulting from shifts in consumer demand presumed to occur throughout the year.

## PRESENT STATUS AND FUTURE PROSPECTS FOR OYSTER CULTURE IN THE PROVINCE OF NEW BRUNSWICK, CANADA

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The 1980 New Brunswick oyster landings amounted to approximately 700 tons, most of which sold to the half-shell trade. The oyster culture sector comprises 723 private leases covering 2,619 acres of seabed. The combined production potential of the culture industry exceeds 23,000 tons annually. Seed shortage is a most important factor limiting development as culturists rely primarily on oysters from contaminated beds, and on seed oysters collected in the shallow areas of the public oyster grounds to stock their leases. Since 1971, spat collection has provided increasing amounts of seed as culturists have successfully used a European collector and mechanized various operations associated with seed production. This paper describes the technology, presents recent results, and discusses implications for the future.

EARLY LIFE HISTORY OF THE OCEAN QUAHOG  
*ARCTICA ISLANDICA* (LINNÉ)

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The larval and early postlarval stages of the ocean quahog *Arctica islandica* were cultured under experimental laboratory conditions. Mature eggs were stripped from ripe adults (obtained off the coasts of Rhode Island and New Jersey during August and September), and were exposed to a 3% solution of 0.1N NH<sub>4</sub>OH for various lengths of time prior to addition of stripped sperm. The larval clams were reared through settlement and metamorphosis using the Wells-Glancy (centrifuged, incubated seawater) method of algal culture and/or modifications of standard hatchery techniques developed by Loosanoff and Davis. Experimental cultures were maintained at various controlled temperatures ranging from 8.5 to 14.5°C. The minimum time to settlement was 32 days at temperatures of approximately 13°C; settlement was not observed in a culture maintained between 8.5 and 10.0°C until approximately 55 days after fertilization. Larval growth rates were significantly lower in the culture maintained at 8.5 to 10.0°C than in cultures maintained between 11.0 and 14.5°C. The smallest shelled veliger observed had length and height dimensions of 95 and 75 µm, respectively. As shell lengths reached 150 to 165 µm, the hinge-line was obscured by the appearance of a low, rounded umbo. The smallest metamorphosing larva observed in any of the cultures had a shell length of 240 µm. Prodissoconch II lengths, as determined from measurements made on 100 early postlarval specimens, ranged from 232 to 289 µm ( $\bar{X}$  = 259.3 µm; SD = 13.1 µm). Optical and scanning electron micrograph sequences of larval and early postlarval stages have been assembled to facilitate identification of specimens of *Arctica islandica* isolated from plankton samples. Juvenile specimens obtained from eggs fertilized in September were reared using a combination of closed-system laboratory tanks and outdoor flow-through systems; shell lengths of these juveniles ranged from 1,005 to 3,940 µm by the following May.

## ANALYSES OF BIVALVE LIFE HISTORIES WITH AN APPLICATION TOWARDS RESOURCE MANAGEMENT

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Approximate life tables for four species of commercially important bivalves, *Mya arenaria*, *Mercenaria mercenaria*,

*Crassostrea virginica*, and *Ostrea edulis*, were constructed from data in the literature. With the exception of *Mya arenaria*, life tables did not exist in the literature and data had to be collated from a large number of studies that often had been performed in different geographical areas. The life history strategies (age-specific mortality and fecundity) were analyzed using the Leslie Matrix projection model. Average allowable larval mortality rates were estimated to range from approximately 99.9% for *M. arenaria* to 99.9999% for *C. virginica*. The reproductive values for all species peaked early and remained high throughout life. The stable age distribution and reproductive values were used to analyze the sensitivity of the population growth rate to age-specific changes in fecundity and survival. For all species and all age classes, the population growth rate was more sensitive to changes in survival than fecundity. Changes in the survivorship of juveniles have as much as two orders of magnitude more effect than proportional changes in adult survival. These analyses were applied toward the assessment of different harvesting strategies because harvesting represents change in age-specific survival. A variety of harvesting strategies were simulated, each concentrating on different age classes and each removing the same total number of clams. The strategy that concentrated harvesting on the youngest harvestable age had the greatest effect on the population growth rate. However, the difference between that strategy and one that removed primarily older individuals was minor. The importance of decreasing mortality of juveniles to increase shellfish abundance has been indicated by the fact that decreasing juvenile survivorship of *M. arenaria* by 50% has approximately the same effect as removing 97% of the adult population.

#### A COMPARISON OF THE ENERGETIC COSTS OF LARVIPARITY AND OVIPARITY IN OYSTERS

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Marine ecologists often discuss the reproductive strategies of benthic invertebrates in terms of an r-K model, a model that involves, among other things, a comparison of oviparity and larviparity, relative fecundity in the adults, planktotrophy and lecithotrophy in the larvae, and the duration of larval development. Published works on commercially valuable oysters, Genera *Crassostrea* and *Ostrea*, allow a quantitative evaluation of the r-K model. This evaluation, however, is not limited in its application only to a theoretical ecological model; indeed, the same evaluation is applicable directly to hatchery conditioning of oyster broodstock. The

present paper discusses a comparison of reproductive energy costs in oviparous *Crassostrea* species and larviparous *Ostrea* species in terms of time and temperature dependence of maturation, energy partitioning between somatic and gonadal tissue, tissue growth versus shell growth, larval production versus adult energy requirements (including feeding during conditioning), and single versus multiple spawning events per reproductive season.

#### SHIPWORM NUTRITION: A REVIEW OF RECENT WORK WITH AN UNUSUAL BIVALVE

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The Teredinidae are unusual among the marine bivalve molluscs in that the adults bore into, ingest, and digest wood in the marine environment. They are equipped with rasping valves, digestive diverticula specialized for wood digestion and, with the exception of *Kuphus*, a wood-storing caecum. The cellulose structure of wood provides a dietary carbohydrate source following cellulolytic activity. The origin (endogenous or exogenous) and site of function of the cellulases responsible for wood degradation are still a subject of discussion. Wood is low in nitrogen content. The dietary nitrogen requirement of the adult teredinid is probably fulfilled from a combination of sources including phytoplankton and bacteria retained during filter feeding, and the utilization of both dissolved organic material (DOM) and dissolved inorganic nitrogen (DIN). The utilization of DIN appears to be related to the presence of symbiotic bacteria in the Gland of Deshayes in the teredinid gill. These microaerophilic bacteria are capable of both fixing nitrogen and producing enzymes which degrade cellulose, and probably play a significant role in both nitrogen and carbohydrate metabolism in their host.

#### HARD CLAM (*MERCENARIA MERCENARIA*) MARICULTURE IN SOUTH CAROLINA: A COMMERCIAL SCALE DEMONSTRATION PROJECT

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A cooperative project to establish a commercial-scale hard clam mariculture facility was initiated in August 1980,

by the State of South Carolina (Marine Resources Research Institute), the National Office of Sea Grant (South Carolina Sea Grant Consortium), and private industry (Trident Sea-farms Company). Results from previous investigations on hard clam culture in South Carolina indicated a reasonable economic potential for intensive culture on a commercial scale. To assess this potential, and to provide realistic analysis at an appropriate scale, this demonstration facility was established near Charleston, SC. The project operated on a three-stage growout protocol: raceways were used to provide initial growout (to 10 mm) and to allow acclimation and disease monitoring for imported seed; intermediate and final growout were accomplished in intertidal and floating field units which provided protection from predators and substrate for support and orientation.

Commercial hatchery seed, ranging in size from 250  $\mu\text{m}$  to 10 mm, were held in raceways for growth, observation, and experimentation. They were transferred to intermediate field growout units (6- $\text{m}^2$  covered cages) at densities of 2,150 to 12,900 clams  $\text{m}^{-2}$  as they acclimated or grew to minimum field planting size. Clams were redistributed, as they attained a mean size of 25 to 30 mm, to final field growout units at densities ranging from 550 to 2,150 clams  $\text{m}^{-2}$ . Using this protocol, over 3.9 million seed were planted in South Carolina estuaries during the initial 15-month planting cycle (October 1980-December 1981). Raceway systems were monitored for growth and survival, and correlations were made between those parameters and certain aspects of water quality (chlorophyll a, total organic carbon, total suspended solids, temperature, salinity, etc.). In addition, the raceway system was used routinely for comparisons of various seed sizes, densities, and water flow interactions. Preliminary results indicate high survivals in both raceway and field growout systems. Growth, while subdued during the late fall and winter, has remained constant and appears to be density-independent at least to a population mean size of 20 mm. Descriptive data on the raceway system, field units, and specialized support equipment are provided.

**AN INEXPENSIVE COMMERCIAL-SCALE NURSERY  
SYSTEM FOR JUVENILES OF  
*MERCENARIA MERCENARIA***

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The state of South Carolina, in cooperation with private industry and the National Office of Sea Grant, has

established a commercial-scale mariculture facility to assess the economic potential of intensive hard clam culture in the southeast. A primary element of this facility is a nursery system which utilizes raceway culture for initial seed grow-out. Incorporating this system into the facility allows for the purchase of smaller-than-required-size seed for field planting (a logistical and economic consideration), provides holding facilities for imported seed for limited quarantine, acclimation, and production control, and provides facilities to generate information on growth and survival of various size seed in relation to water flow, seed density, and water quality. The system consists of eighteen 2.7- $\times$  1.2- $\times$  0.1-m (2.7  $\text{m}^2$  effective bottom area each) and twenty 4.8- $\times$  0.6- $\times$  0.1-m (2.6  $\text{m}^2$  effective bottom area each) raceways each supplied with high salinity water (28 to 30 ppt at 38  $\ell \text{min}^{-1}$ ) through either a gravity-fed header box system or through direct plumbing to pump transmission lines. Growth and survival of raceway populations are monitored weekly, and a complete census is performed every 3 months. In addition, chlorophyll a and organic carbon striping rates are determined randomly; basic hydrographic parameters (temperature and salinity) are monitored daily. Results from the initial 9 months of operation indicate that rapid growth and high survival are characteristic to the system. Total dependent biomass increased by 92%, and survival averaged over 95% in all size classes during the first census period (October-December 1980). The second census (March 1981) indicated a biomass increase of almost 200% (relative), and survival rates of over 80%. Striping rates of chlorophyll a ranged from  $< 2\%$  to  $> 60\%$  at water flows of 34 to 40  $\ell \text{min}^{-1}$ . Striping rates were directly correlated to initial water quality, temperature, and seed size/density relationships. Raceway construction details, materials list, and cost summaries are provided.

**UPTAKE OF KEPONE FROM SEDIMENTS IN SUSPENSION  
BY THE OYSTER *CRASSOSTREA VIRGINICA* (GMELIN)  
AND THE WEDGE CLAM *RANGIA CUNEATA*  
(SOWERBY) IN LABORATORY AND  
FIELD STUDIES**

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Oysters (*Crassostrea virginica* [Gmelin]) and wedge clams (*Rangia cuneata* [Sowerby]) were subjected to Kepone contamination in laboratory trays receiving sediment suspensions prepared with contaminated sediments from the James River at the Virginia Institute of Marine

Science, in pier trays receiving water pumped directly out of two tributary creeks of the James River, and in wire trays laying on the bottom of the James River. Both species took up Kepone very rapidly and equilibrium with ambient concentrations was attained within 1 week. The concentration in animal tissues after exposure for 1 week was directly related to the concentration of Kepone in the suspensions for the same week. Depuration of Kepone by oysters was also rapid, especially during the first week, but the rate of depuration decreased as the tissue concentration was reduced. The high kinetics of Kepone exchange between oysters and their environment make them excellent indicators of changes in ambient concentrations of Kepone. Concentration factors in the tissues of oysters ranged from 574 to 5,625 in laboratory trays, and from 5,555 to 27,778 in pier trays; in the wedge clam, they ranged from 521 to 2,423 in laboratory trays, and from 12,857 to 42,857 in pier trays. Concentration factors were inversely related to concentrations in the sediment suspensions. The data suggested that a substantial amount of Kepone may have been picked up from solution. Oyster feces produced by oysters receiving contaminated sediment suspensions contained several times more Kepone than did pseudofeces or sediments settling out by gravity.

#### HEMOLYMPH CONSTITUENTS OF NORMAL AND PROCTOECES MACULATUS-INFECTED BLUE MUSSELS (*MYTILUS EDULIS*)

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Hemolymph components of normal blue mussels and those infected with the fellodistomatid trematode *Protoeces maculatus* Looss were monitored over an annual cycle. Hemolymph carbohydrate content of the normal and infected mussels showed a marked temporal variation with peak concentration occurring in July. The magnitude of the peak for the infected animals was much greater than the normal mussels. Hemolymph protein was reduced in infected mussels relative to that of normal individuals. Total free amino acid content of normal and infected mussels was similar but normal individuals underwent significant fluctuations in the concentration of some of the major amino acids (taurine, alanine, glycine, serine) while infected mussels maintained more constant levels.

#### GROWTH RATE ANALYSES OF *MYA ARENARIA* USING ALIZARIN-STAINED CHONDROPHORES: A NEW TECHNIQUE

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The growth rate of alizarin-stained juveniles of *Mya arenaria* Linné is useful for site selection in on-bottom culture of planted seed or it may be correlated with environmental or organismic variables in ecological studies. In *M. arenaria*, growth in chondrophore length, defined as the maximum distance from the umbo to the distal margin of the chondrophore, is related to shell length by the least squares regression line: shell length (mm) = 6.71 chondrophore length (mm) + 5.73 mm, and N = 300, and R<sup>2</sup> = 0.94. This equation holds through sexual maturity but does not apply to slow-growing, thick-shell clams collected from the upper intertidal zone in coarse sediments. For example, in 67 of *M. arenaria* collected from coarse sediments in the upper intertidal zone in central Maine, the shell length = 4.26 chondrophore length (mm) + 12.9 mm.

When fast-growing juvenile clams are immersed in 1  $\mu$ m-filtered seawater containing 25 ppm alizarin red S (sodium alizarin monosulfonate) and fed cultured algae commensurate with clearing rates for a period of 3 days at 10 to 20°C, a distinct red line is formed on the growing edge of the chondrophore. Clams are planted in desired field locations at constant densities and recovered after a constant growth period. Clams are then shucked, the left valve is removed, and the chondrophore is measured under a dissecting microscope with the shell oriented in putty and the chondrophore growth axis parallel to the stage.

The chondrophore growth increment is determined for each shell by subtracting  $L_{initial}$  (length from the umbo to the red stain line) from  $L_{final}$  (length from the umbo to the distal margin of the chondrophore). Each growth site is thus characterized by a mean chondrophore growth increment, and sites may be compared using analysis of variance and a multiple range test.

The advantages of this technique include the following: (1) each clam is marked with an unmistakable red line which distinguishes it from wild field populations; (2) if cultured algae is available, thousands of clams may be marked at the same time; (3) no effort is wasted determining the initial length of clams which are subsequently lost in the field (due to predation, etc.), because all the

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recovered clams have the L<sub>initial</sub> conveniently marked on their hinge; and (4) alizarin-staining apparently has no significant effects on growth rate in *M. arenaria*.

#### ENVIRONMENTAL AND GENETIC INFLUENCES ON THE REPRODUCTIVE CYCLE OF THE BLUE MUSSEL *MYTILUS EDULIS* LINNÉ

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There have been numerous studies of reproductive cycles in marine bivalve molluscs, including the blue mussel *Mytilus edulis*. The results of these spatially and temporally separated studies of different molluscs frequently have been synthesized. One resulting generalization from that synthesis is that spawning generally is earlier in temperate water species at the equatorial edge of their range due to earlier warming of the water.

The results of our study showed there was no clear influence of latitude on the reproductive cycle of populations of *M. edulis* from the northeastern coast of North America. Two populations, one on the north shore and one on the south shore of Long Island, NY, exhibited the largest difference in reproductive cycle of all populations studied, even though the environmental water temperatures essentially were identical. Instead, the differences in reproductive cycle appeared to be due to variations in the period of maximum food availability at each location which, in turn, affected the acquisition and storage of nutrient reserves for vitellogenesis. The results of transplant experiments indicated there may be a degree of genetic control over the reproductive cycle in *M. edulis*.

#### SUSCEPTIBILITY AND RESISTANCE OF THE ROCK CRAB *CANCER IRRORATUS* SAY TO NATURAL AND EXPERIMENTAL BACTERIAL INFECTIONS

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The incidence of naturally occurring bacterial infection in a Connecticut population of the rock crab *Cancer irroratus* Say varied between 10 and 60%. Fluctuation in incidence

was correlated significantly with hemocyte concentrations. Twelve percent of the crabs sampled during January were found to be infected with a previously undescribed *Vibrio*. The *Vibrio* was demonstrated as pathogenic over a wide range of temperatures. High mortalities in experimentally infected crabs appear to result from hemocyte clumping and extensive intravascular clotting triggered by an endotoxin.

The lobster pathogen *Aerococcus viridans* var. *homari* was inoculated experimentally into the host and was found to be pathogenic at 25°C to *C. irroratus*, a prey species of the lobster. Results suggest that this species can serve as a reservoir host for *A. viridans* var. *homari*.

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#### EFFECTS OF REDUCED SALINITY ON EARLY DEVELOPMENT OF PROSOBRANCH GASTROPODS

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Gastropods of many marine species deposit their fertilized eggs intertidally, enclosing the eggs within complex encapsulating structures. The developing embryos of these intertidal species are, therefore, potentially exposed to a variety of intertidal stresses, including that of exposure to low salinity during rainstorms. The effect of a given salinity decrease on embryonic development is difficult to define precisely, in part because of the well-known interactive effects of temperature. In addition, the rate of salinity change appears to be a major determinant of stress tolerance for a number of prosobranch gastropod species. Experiments suggest that the egg capsules of *Hyanassa obsoleta*, *Thais lamellosa*, and *Thais lima* reduce developmental mortality in the face of reduced ambient salinity by decreasing the rate of change, rather than altering the final magnitude of the drop in osmotic pressure of the intracapsular fluid. The present-day gastropod egg capsule may have evolved, at least in part, as an adaptation to fluctuations in intertidal salinity.

#### GAMETOGENESIS AND GROWTH OF THE PACIFIC OYSTER *CRASSOSTREA GIGAS* (THURNBERG) STOCKS IN TWO BAYS IN THE STATE OF WASHINGTON

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As is true with many bivalves, the reproductive cycle dominates the life of the Pacific oyster *Crassostrea gigas*.

Extensive gonad proliferation occurs in environments

characterized by warm ( $\geq 20^{\circ}\text{C}$ ) temperatures and high productivity. Condition indices typically exceed values of 16 under these conditions with gonadal products occupying 40 to 50% of the dry tissue weight.

Whereas some bivalves partition energy reserves to different areas (e.g., shell deposition, gonadal tissue, somatic tissue, etc.) during gametogenesis, the Pacific oyster appears to direct an extensive amount of energy toward the annual reproductive cycle.

A comparison of two different gametogenic patterns in two different bays revealed how shell growth, as one energy partition, was dependent on the gonadal cycle in the Pacific oyster, and not upon either temperature or food conditions, as in some other bivalves. A comparison of these events with other bivalves is presented.

#### RECENT ECONOMIC TRENDS IN THE MOLLUSCAN COMMERCIAL FISHERIES OF THE SOUTH ATLANTIC STATES

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During the 1950's and 1960's, commercial oyster (*Crassostrea virginica*) harvesting dominated the estuarine molluscan fisheries of the south Atlantic coast of the United States. During the 1970's, hard clam (*Mercenaria* spp.) harvesting, especially in the Carolinas, increased dramatically as south Atlantic producers responded to rising United States prices. South Atlantic hard clam producers have recently entered the mainstream of the United States market. The mean real ex-vessel price of hard clams has risen from \$0.45/lb of meat in the 1960-1969 period to \$0.75/lb in the 1970-1979 period in the south Atlantic. In contrast, south Atlantic oyster prices have not increased in real terms since the 1950's. The supply response between states to the changing prices has varied because of differences in environmental factors, institutional frameworks, and industry structures which are discussed.

#### ENVIRONMENTAL REGULATION OF MOLLUSCAN REPRODUCTION

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The patterns of reproductive periodicity for different species of bivalve molluscs vary both regionally and geographically. Generally, species of bivalves reproduce annually, semi-annually, or continuously in different environments of the coastal and estuarine regions. Factors regulating the species-specific patterns of reproduction to the environment are of interest to basic ecology and evolution, and also for practical applications. Recent studies show that a continuous interaction between internal factors (i.e., age, metabolism, and somatic growth), and external factors (i.e., temperature and food availability), and their coordination through neurosecretory factors regulates the reproductive cycle. A complex functional integration of reproduction to the environment at a given time and over time seems to specify when energy may be diverted for gametic production and, hence, the timing of this event in the reproductive cycle. Varying degrees of synchronization and coordination of the events of reproductive cycle among members of the population seem to produce the observed pattern for species in different environments.

#### DISPERSAL AND RECRUITMENT OF TROPICAL MUSSEL LARVAE AS AFFECTED BY TEMPERATURE AND SALINITY

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Over a period of 3 years, larvae of three populations of each of two species of tropical mussels, *Perna perna* and *Perna viridis*, were reared through metamorphosis over a broad range of temperatures and salinities. Data were collected on fertilization, survival, growth, onset and delay of metamorphosis, and shell deposition.

In the laboratory, larvae of all populations of both species responded similarly, tolerating a wide range of temperatures (mean:  $25$  to  $26^{\circ}\text{C}$ ) and salinities (mean: 29 to 31 ppt). Normal fertilization was possible over a wider range of conditions than was embryogenesis. Older larvae tolerated a broader range of temperatures and salinities than did embryos and early larvae. Critical periods for survival involved the transition to planktotrophy 24 to 48 hours after fertilization, and the onset of metamorphosis 11 to 13 days after fertilization. Optimal conditions for growth were much warmer than optimal conditions for survival. Sublethal salinities adversely affected feeding rates while supraoptimal temperatures reduced the duration of feeding stages, both resulting in smaller larvae at completion of metamorphosis. At sublethal temperatures and salinities,

resorption of the velum occurred soon after onset of metamorphosis, thus preventing the pediveliger from feeding and limiting the duration of the delay of metamorphosis. Abnormally small veligers with deformed shells were most common at low salinities combined with high temperatures.

Prodissoconch II shells of larvae reared at 14 ppt were significantly thinner than those deposited at 28 or 42 ppt. If thicker shells are stronger, larvae growing at near-oceanic salinities will metamorphose with stronger shells. At warm temperatures and, hence, high growth rates, growth ridges were spaced more widely suggesting that the process causing ridging is a function of time more than growth rate. Ridges were produced in these species at 11.8- to 12.6-hour intervals at both 17.9 and 27.5°C. Assuming that closely spaced growth ridges reinforce the larval shell more than widely spaced ridges, larvae which grow more slowly will metamorphose with stronger shells.

Dry weight percentages of magnesium in whole pediveliger shells were highest in shells deposited above 24°C, and lowest in those deposited at 14 ppt. The relatively high magnesium content found in these aragonitic larval shells may result in weaker or more soluble shells.

Responses of these larvae to variations in temperature and salinity may affect dispersal and recruitment of the adult mussels. The temperature and salinity characteristics, movement, and persistence of the water mass in which larvae are dispersed affect the physiological processes that determine (1) the ability and opportunity to feed, (2) the duration of planktonic life which affects potential for dispersal and exposure to planktonic mortality factors, (3) the ability to select sites for settlement, and (4) shell morphology and physiological status at metamorphosis, which may alter susceptibility of early postlarvae to benthic mortality factors.

#### HIGH-DENSITY HATCHERY PRODUCTION OF JUVENILES OF THE QUEEN CONCH *STROMBUS GIGAS* LINNÉ

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In Florida, the Bahamas, and many areas of the Caribbean, populations of the queen conch *Strombus gigas* Linné are overfished. Catches and exports to the United States have declined substantially in recent years. The objective of this continuing study is to develop methods for high-density hatchery production of queen conchs for reseeding depleted natural populations (extensive mariculture) or for captive growout to market size (intensive mariculture).

Experimental procedures developed during the 1980 spawning season have enabled us to rear several hundred larvae through metamorphosis for the first time under specific laboratory conditions. Refinements to our procedures are anticipated, especially those that will permit more cost-effective high-density stocking rates of larvae ( $> 500 \text{ } \ell^{-1}$ ). Results from more than 500 experiments with 156 cultures of larvae have identified important factors affecting production of juvenile conchs. Tentatively we conclude that the following aspects of our methods are important: (1) disinfection of egg masses, (2) eggs hatched in deep, aerated vessels in which larvae are reared, (3) mixed species diet of warm water phytoplankton first provided 24 to 48 hours after hatching, (4) complete daily water exchanges begun 100 to 120 hours after hatching, and (5) provision of suitable substrates for metamorphosis (film of epiphytic diatoms) 25 to 30 days after hatching.

#### SENSITIVITY OF JUVENILE HARD CLAMS (*MERCENARIA MERCENARIA*) TO AMMONIA

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Lethal and sublethal renewal bioassays were performed with larval and juvenile hard clams (*Mercenaria mercenaria*) up to 10 mm in length. Lethal ammonia concentrations (30-day  $TL_m$ ) were determined for 4-, 6-, and 10-mm animals as 20.0, 28.0, and 34.5 ppm  $\text{NH}_3 + \text{NH}_4^+$ , respectively. Sublethal bioassays examined growth rate reduction, food removal, and larval development.

#### CONTAINERIZED RELAYING OF POLLUTED OYSTERS IN MISSISSIPPI SOUND: A SUMMARY

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Plastic containers constructed of polyethylene structural foam were used to cleanse commercial quantities of polluted oysters. During five experiments, chicken coops (86 × 56 × 20 cm, Piper Industries, Jackson, MS) with solid bottoms and hinged lids, containing one sack (0.029 m<sup>3</sup>) of oysters each were arranged in 3-coop stacks and suspended in approved shellfish-growing waters (above the bottom and below the mean low tide level). Oysters purged fecal coliforms from initial MPN values as high as 14,000/100 g

to the recommended 50 MPN/100 g during 15 days, barring failure of the suspension rope.

Piper coops were also used in an onbottom, longline-relaying system. In three experiments, three coops were placed individually on firm or soft substrates, and connected to a common longline of 1.25-cm (diameter) nylon rope. Oysters purged fecal coliforms from 7,400 MPN/100 g initially to 50 MPN/100 g; however, complete success was limited by sediment-fouling problems. A firm, shell substrate is required because the coops tend to settle into soft, muddy bottoms.

Plastic coop bottoms (Phillips Petroleum Co., Henderson, KY) were used in three offbottom experiments with an oyster rack. Phillips coops (86 × 56 × 10 cm), which have a foraminated bottom and no lid, also held one sack of oysters each. The rack (3.6 × 1.8 × 1.2-m) (patent, E. R. Gollott, Biloxi, MS), constructed primarily of welded angle iron, held 48 coops in a sliding tray arrangement (6 trays × 2 rows × 4 levels). Oysters from 8 coops, located in different positions and levels in the rack, were tested for fecal coliform bacteria. Initial fecal coliform values as high as 23,000 MPN/100 g were reduced to below 50 MPN/100g within 10 days.

Success of relaying was primarily dependent upon sustained, approved water quality; however, the experiments showed that the container type, the relaying method, and oyster condition were also critical factors. The Phillips coop was suitable for rack-relaying, and resulted in mortalities of only 1.6%, compared to mortalities exceeding 70% using conventional onbottom, relaying techniques. The Piper coop was more suitable for suspension- and longline-relaying. Failure to achieve successful cleansing in one of the longline trials resulted from poor physiological condition of oysters (Condition Index = 4.0 units), that was caused by recent spawning. Holding the oysters off the bottom would reduce recontamination from sediment-borne pathogens. The shallow, flow-through coops permit cleansing of all layers of oysters, eliminating the problem of interior oysters that fail to cleanse.

Containerized relaying has immediate applicability to commercial oyster relaying in Mississippi. Adequate types and usage of containers ensure complete second harvests, and serve as acceptable cleansing, transport, and storage containers throughout relaying and processing.

#### TEMPORAL DISTRIBUTION OF SURF CLAM LARVAE OFF SOUTHERN NEW JERSEY

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Surf clam larvae were sampled along two transects from Cape May and Hereford inlets to Five Fathom Bank over a period from spring 1976 to fall 1978. Two main pulses of high larval concentrations occurred in all 3 years, from May to June and from September to October. Additionally, there were minor peaks in late July 1976, and in early July 1978. Straight-hinged larvae were found as early as 21 April 1977, and large numbers of larvae were still present as late as 13 October 1978.

The main larval peaks were derived from two different populations of adult surf clams, and could be correlated with the temperature regimes of those clams. The spring larval pulses were derived from the inshore population where the temperature of the shallower waters rose faster. The occurrence of larvae in the fall resulted from the spawning of offshore clams at depths below the summer thermocline. As the thermocline breaks down, warmer water reaches the bottom, inducing the clams to spawn. There was no indication that the inshore stocks regenerated gonads through the summer and fall. The minor July peaks could be due to a depression of the thermocline, stimulating clams which had been in cooler water just below the thermocline.

#### PRELIMINARY ANALYSIS OF THE EFFECT OF A COMMERCIAL FISHERY ON THE SIZE STRUCTURE AND SHELL CONDITION IN POPULATIONS OF THE SNOW CRAB *CHIONOECTES OPILIO*

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Snow crabs (*Chionoectes opilio*) have been fished commercially in Newfoundland since 1968. Fishing effort and landings have increased dramatically since inception of the fishery with exploitation rates in some areas reaching 70% and higher. To date, little effort has been expended to determine what affect commercial exploitation has on size structure and shell condition of adult snow crabs. Data on carapace width and shell condition of crabs caught during pre-exploitation exploratory surveys are compared to data obtained from the commercial fishery to determine what effect fishing on a commercial level has on these parameters.

#### SUCCESSFUL TRIAL OF HABITAT COLLECTORS FOR SAMPLING JUVENILE ROCK CRABS (*CANCER* *IRRORATUS*) AND MUD CRABS (*NEOPANOPE TEXANA SAYI*)

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Different types of habitat collectors used to study post-larval stages of rock and spiny lobster *Panulirus* sp. were tested in the Northumberland Strait, New Brunswick, Canada, for their potential use as sampling devices for decapod crustaceans. Types of collectors used were: the square frame, the Witham habitat, the fishing net bag, and artificial seaweed. They were suspended in 2, 5, and 10 m of water, near the surface, at midwater level, and on the bottom. Frames and bags were filled either with mussel (*Mytilus edulis*) shells, plastic tubing, or kelp fronds. Rock crabs (*Cancer irroratus*) and mud crabs (*Neopanope texana sayi*) were the two important species collected. Overall 113 rock crabs were caught in 1979, and 159 rock crabs and 61 mud crabs in 1980. All sizes of juvenile crabs were sampled. Carapace width (CW) ranged from 2 to 56 mm for rock crabs, and from 4 to 19 mm for mud crabs. We noticed a selectivity effect in the frames for rock crabs over 19 mm CW and for mud crabs over 17 mm CW because of the

mesh size used (14.7 mm diagonally).

In decreasing order, the artificial seaweed, the square frame, and the Witham habitat were the most efficient with catch-per-unit-effort (CPUE) of 5.63, 3.54, and 1.54 rock crabs/collector-haul, respectively. They were less efficient for mud crabs. The CPUE and mean size of rock crabs varied with depth and level, indicating their potential for sampling both species in relation to environmental variables such as area, time, temperature, salinity, or substrate type. The small size at which crabs first could be caught also indicates the possibility of using these collectors to evaluate the time, area, and intensity of recruitment from the planktonic to the benthic life stages. Sampling with such collectors is advantageous over the current methods in use like SCUBA diving, manual collection, or groundfish stomach examination. The main advantages are the possibility of simultaneous sampling in time and space, and a greater control over the exact area and time of sampling.

The following abstract was presented at the **1980 Annual Meeting** of the National Shellfisheries Association, in Hyannis, Massachusetts, June 9-12, 1980.

**THE EFFECTS OF SEVERAL DIETARY PROTEIN CONCENTRATIONS AND DIFFERENT LIPID LEVELS ON GROWTH AND DEVELOPMENT OF JUVENILE LOBSTERS (*HOMARUS AMERICANUS*)**

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Juvenile lobsters (*Homarus americanus*) were fed artificial diets containing either 5 or 10% lipid (percent dry weight) and semipurified crab protein at concentrations of

50, 43, 35 or 30%. The diets were made isocaloric by adjusting the content of  $\alpha$ -cellulose. Survival of lobsters whose diets contained 10% lipid was significantly higher than those receiving 5% lipid. While there were no significant differences in weight gain or normalized biomass increase between lobsters fed different protein concentrations within the same lipid category, all test diets containing 10% lipid produced significantly better growth than those on the 5% lipid diets. The findings suggest that the total protein requirements of lobsters may be considerably lower than that previously believed necessary and that 5% lipid is insufficient for optimal growth and survival of juvenile lobsters.



ABSTRACTS OF TECHNICAL PAPERS

*Presented at 1981 Annual Meeting*

WEST COAST SECTION

NATIONAL SHELLFISHERIES ASSOCIATION

Portland, Oregon

September 4–5, 1981



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**COMMENTS ON THE SETTLEMENT OF MANILA  
CLAM SPAT (*TAPES PHILIPPINARUM* [ADAMS  
AND REEVE]) AT FILUCY BAY,  
WASHINGTON, USA**

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At Filucy Bay, WA, population abundance of wild Manila clams ( $\geq 3$  mm shell length) within a 10- $\times$  30-m plastic netting enclosure increased from 20 m<sup>2</sup> to 157 m<sup>2</sup> between April 1979 and October 1980. Abundance of these clams in an open control remained unchanged during the same period. Three hypotheses were proposed to explain the observation: (1) juvenile clams migrated or were washed into the plot from adjacent areas, (2) larval clams settled in the plot subsequent to its construction, and (3) spat from an undetected settlement in 1978 were present on the beach when the enclosure was constructed. Density differences would arise as a result of larvae being attracted or concentrated in the enclosure, or as a result of differential survival between the two areas. The 1978 spat could not be detected in population samples because the minimum screen sizes were too large to retain clams under 2- to 3-mm shell length. Subsequent differences in abundance between enclosure and control areas would be explained as differential mortality related to predation and/or disturbance. Size-frequency analysis indicated that successful settlements occurred in 1976, 1977, 1978, and 1979. Weekly sampling in 1980 demonstrated Manila clam spat settled in Filucy Bay in late August and September, but at low densities. Spat attained a shell length of approximately 500 to 600  $\mu$ m by late October 1980 and, based on earlier data, would reach a size of 700  $\mu$ m by the following spring. Therefore, spat from the 1978 settlement were probably present but too small to be retained in screens (minimum 3-mm mesh) used to process samples taken in the enclosure area prior to its construction. It is apparent that the "increased" abundance of wild clams is a result of reduced mortality and does not represent an actual increase. Reasons for rejection of the alternative hypotheses are discussed.

**DISTRIBUTION, ABUNDANCE, AND GROWTH OF DUNGENESS  
CRAB IN GRAYS HARBOR: THE RELATIVE IMPORTANCE  
OF ESTUARINE POPULATIONS AND POTENTIAL  
IMPACT OF DREDGING**

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Coastal estuaries are considered important habitat for early life-history stages of *Cancer magister* Dana and may constitute nursery areas through provision of abundant food and reduced competition with older year-classes. Perturbations of major estuaries could reduce juvenile populations to such an extent that recruitment to offshore fisheries is reduced; such events may account for the long-term decline of the San Francisco crab fishery.

A proposed widening and deepening program for Grays Harbor ( $19.4 \times 10^6$  yd<sup>3</sup> of sediment to be removed) prompted this study of spatial and temporal changes in crab abundance. Nine stations were sampled by trawl twice to once a month from May 1980 to July 1981. Area swept by the net was calculated and used to derive crab densities per 100 m<sup>2</sup>. These values were extrapolated to population estimates for regional areas about each station.

Young-of-the-year crabs enter Grays Harbor in early April at about 7-mm carapace width and may reach a maximum of 70 mm, depending on time of metamorphosis, one year later ( $\bar{X}$  = 50 mm). Crabs of 1+ years constituted  $> 65\%$  of total numbers caught on an annual basis which, for spring and early summer, highlights inefficiency of gear in sampling early instars. Estimates of total crab populations in Grays Harbor, based solely on actual trawl catches, are 11.57, 6.51, and 1.59 million crabs for spring (March–May), summer (June–August), and winter (September–February), respectively. When corrected for inefficiency of gear and deletion of a single, spurious trawl, population estimates are 10.46, 54.00, and 3.18 million crabs in spring, summer, and winter, respectively. (The huge increase in the summer estimate reflects very inefficient net sampling of first and second instars.) Crabs were significantly more abundant in the outer than inner harbor, and more abundant in summer than winter.

Entrainment rates by hopper dredges varied from 0.22 to 0.47 crabs/yd<sup>3</sup> in the outer harbor to 0.03 to 0.11/yd<sup>3</sup> in the inner harbor. Correcting entrainment to actual mortality, estimates of total crabs killed during widening and deepening are 2.53 million during a combined summer/winter schedule, and 1.35 million during winter only. This mortality could constitute from 5% of the total estimated summer population to 42% of that in winter. Whether this loss would eventually constitute a severe impact to the fishery offshore of Grays Harbor is difficult to predict, because no comparative data exist on the relative proportions of an incoming year-class that enter the harbor or permanently remain offshore after metamorphosis.

SETTLEMENT AND SUBSEQUENT SURVIVAL OF  
COMMERCIALY-REARED EYED-PEDIVELIGER  
LARVAE OF THE PACIFIC OYSTER  
*CRASSOSTREA GIGAS* (THURNBERG)

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A series of factorial experiments were conducted using eyed-pediveliger oyster larvae (*Crassostrea gigas* [Thurnberg]) reared at a commercial hatchery in Netarts, Oregon.

Experiments on the combined effects of temperature and salinity indicated that temperature had a highly significant effect on the setting of Pacific oyster larvae. At temperatures from 15° to 30°C, the percentage of larvae setting during a 48-hour period increased as temperature increased. There was no significant difference in setting at salinities from 15 ppt to 30 ppt, and there was no evidence of a temperature-salinity interaction. In the one experiment carried out with Kumamoto larvae (another variety of *C. gigas*), setting differences among the temperature and salinity combinations were statistically insignificant.

Providing the larvae with 120,000 cells/ml of the alga *Pseudoisochrysis paradoxa* did not significantly improve setting during a 48-hour period at any of the temperatures tested (15-30°C).

Storing eyed larvae at 5°C for 5 to 8 days before setting appeared to increase the percentage of attaching larvae. The best temperature for setting stored larvae was 25°C.

Larvae that attached to oyster shells during the setting experiments were held 8 to 9 months in a tank of raw seawater in the laboratory. Mortality appeared higher for larvae that had been stored for several days before being set; however, these experiments should be repeated under more natural conditions before any conclusions are drawn regarding spat survival.

ADVANCES IN CULTURE OF THE PURPLE-HINGE  
ROCK SCALLOP

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The purple-hinge rock scallop *Hinnites multirugosus* (Gale) is of regional importance on the west coast of the United States and Canada, and has been considered a prime candidate for marine aquaculture in recent years. Prerequisite

to commercial mariculture is a sound understanding of the nutritional requirements of the early developmental stages. The primary focus of our research has been to investigate and define optimal diets for larval, post-larval, and juvenile stages. The uptake of finely divided particulate matter using radioisotopes was also investigated.

Utilizing a multichambered environmental control system, six algal diets (*Isochrysis galbana*, *Monochrysis lutheri*, a Tahitian strain of *Isochrysis* sp., *Rhodomonas* sp., *Carteria pallida*, and a mixture of *Rhodomonas*, *Monochrysis*, and *Isochrysis*) were fed to scallop larvae each at five concentrations. Two separate experiments were run utilizing algae harvested in exponential and stationary growth phases. Characteristically, algae harvested in the stationary phase are high in lipid, while those harvested in the exponential phase are high in protein, as percent dry weight. The data clearly show the superiority of *Isochrysis* and T-*Isochrysis* sp. The *Isochrysis* diet supported over 80% survival through metamorphosis at a concentration of  $2 \times 10^5$  cells/ml. The significant separation between the stationary and exponential *Isochrysis* diets demonstrates the importance of lipid to the developing larvae. It is proposed, and presently under investigation, that a diet with a high percent accessible fatty acids (triglycerides) may enhance the storage of high-energy lipids required by the developing larvae to undergo metamorphosis.

Post larvae were treated in a similar manner and subjected to five diets: *I. galbana*, T-*Isochrysis*, *M. lutheri*, *Thalassiosira pseudonana*, and *Phaeodactylum tricornutum*. Results show the continued success of *I. galbana* and T-*Isochrysis* at concentrations between  $1 \times 10^5$  and  $2 \times 10^5$  cells/ml. The other diets supported minimal growth.

Groups of 15 juveniles of *Hinnites* (10 mm) were supplied equal rations by packed cell volume (0.05 ml/day) of T-*Isochrysis*, *Tetraselmis suecica*, *Dunaliella salina*, and a mixture (1:1:1) of these three. Experiments were conducted in the bivalve culture laboratory at Scripps Institution of Oceanography. Results yielded a mean shell diameter increase for the 1:1:1 mixture of  $2.1 \pm 0.5$  mm. *Tetraselmis suecica* and T-*Isochrysis* supported only  $1.6 \pm 0.8$  mm and  $1.1 \pm 0.8$  mm, respectively. *Dunaliella salina* supported only minimal growth.

The uptake of particulate organic matter was investigated using radiolabeled abalone feces fed to juveniles of *H. multirugosus* for one week. Data indicate that juveniles assimilate finely divided particulate matter as food. Significant accumulation occurs in DNA, RNA, protein, carbohydrate, free-reducing substances, and most dramatically in the lipid fractions.

In all feeding runs, except juveniles, algal concentrations in excess of  $5 \times 10^5$  cells/ml were detrimental. *Isochrysis galbana* and T-*Isochrysis* sp. proved overall to be the

optimal diets at the specified concentrations. *Monochrysis lutheri* showed toxicity at stationary concentrations. Juvenile rock scallops do assimilate fine particulate organic matter.

**A MODEL TO EXPLAIN THE INDUCTION OF SETTLEMENT  
AND METAMORPHOSIS OF PLANKTONIC EYED-  
PEDIVELIGERS OF THE BLUE MUSSEL  
*MYTILUS EDULIS* L. BY CHEMICAL  
AND TACTILE CUES**

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The mussel *Mytilus edulis* is a commercially important species worldwide and is becoming so in the United States. Although the potential for continued development of the mussel culture industry in the United States is good, continued expansion is inhibited by the lack of an efficient method for larval recruitment. The identification of a simple chemical inducer of settlement and metamorphosis of planktonic mussel pediveligers could provide an inexpensive, easily controlled, and reliable method for recruiting larvae onto a substrate in a controlled setting system. The focus of this research has been to determine the role of chemical cues in the settlement and metamorphosis of planktonic pediveligers of *M. edulis*. The suggested general role of chemical cues during the settlement of planktonic pediveligers of other bivalves was compared to the results obtained for mussel larvae.

Aliquots of uniformly competent, hatchery-reared pediveligers were tested in culture dishes with smooth glass surfaces for settlement onto a variety of marine algae collected from Humboldt Bay, CA. Of the species which were tested, the pediveligers settled in high numbers (>30%) only onto the red filamentous alga *Platythamnion villosum* (Ceramiales). The induction of larval metamorphosis was ascertained by direct observations of the morphology of the post larvae approximately 40 hours following attachment to the algal substrate.

Pediveligers were tested in a similar manner following exposure to extracts of *P. villosum* and to simple amino acids. The larvae settled and metamorphosed in response to intact *P. villosum* (58.9%) and to the precipitate (48.2%) and supernatant (14.5%) obtained following centrifugation of a seawater homogenate of *P. villosum*. In addition, the larvae settled and metamorphosed in response to L-3,4-dihydroxyphenylalanine (L-DOPA) at concentrations of  $10^{-6}$  M (12.2%) and  $10^{-5}$  M (36.9%), but not in response to gamma-aminobutyric acid at the same concentrations, nor in controls.

These results are striking in reference to the occurrence

of lanosol (2,3-dibromo-4,5-dihydroxybenzyl alcohol), a compound structurally similar to L-DOPA, in the cuticle of red algae of the order Ceramiales. In addition, proteins containing terminal catecholic groups (1-substituted-3,4-dihydroxycatechols) are precursors in the general process of quinone tanning. Mussel pediveligers are found to settle in high numbers onto hydroids which are covered with a perisarc, a quinone tanned structure. I suggest that *M. edulis* pediveligers will settle, attach and metamorphose in response to phenolic compounds, particularly those containing catechol groups.

The shell proteins and the adhesive used for attachment by bivalves and barnacles are also formed, through 1-substituted-3,4-dihydroxycatechol precursors, by the process of quinone tanning. The catechol groups in the adhesive may directly induce metamorphosis in a variety of bivalve and barnacle larvae. Secretion of the adhesive may also trigger the onset of moulting in barnacle larvae. Previous findings suggest that in oysters the periostracal protein enhances the setting of pediveligers. A model is proposed to explain how chemical and tactile cues are integrated during settlement and in the choice of an attachment site for bivalve pediveligers.

A variety of current findings indicate that these results are of general ecological significance. The model provides a mechanism which can explain the overall recruitment strategy of mussel pediveligers into intertidal landscapes. The adaptive significance may not be in accrued benefits associated with a gregarious habit, but may reflect a recruitment strategy which confers increased interference, competitive abilities (e.g., inhibition) to a population in a discrete area. In addition, these findings provide a general mechanism to explain the recruitment of predators, such as sea stars, into areas of preferred prey, such as mussel beds.

These results hold great promise for manipulating the behavior of bivalve larvae during the settlement process. Ultimately, application of these results may allow for a very high settlement success in controlled setting systems.

**AGGREGATIONS OF THE JAPANESE OYSTER DRILL  
*OCENEBRA INORNATA* (RECLUZ)**

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The Japanese oyster drill *Ocenebra inornata* is an exotic muricid gastropod which was imported from Japan into Washington state on seed oysters in the early 1920's. It has been a problem to oyster growers in many areas of Puget Sound despite control techniques such as disking fallow

oyster beds, draining standing salt water pools, handpicking, and chemical treatments such as Sevin. Preliminary work is being performed to define pheromones excreted by oyster drills as cues for mating and egg laying aggregations which occur during early spring and fall. These aggregations are usually easily distinguished from aggregations caused by environmental conditions (shading, water currents, etc.) or feeding (several snails eating a gaping oyster).

Fifty-four artificial nesting sites, consisting of cardboard beer-carton dividers dipped in a concrete-sand mixture, were monitored on a biweekly schedule in Rocky Bay (Case Inlet of Puget Sound) from late April to late August. The number of oyster drills increased over the summer with an increasing proportion of younger drills (< 10 mm shell length). The number of egg layers and aggregations decreased to zero by late May and remained stable in number throughout the summer.

Ten aggregations (120 animals) from another Puget Sound site in Mud Bay were analyzed for activity or relative location with the aggregation. Low percentages of the total number of drills were represented by feeding individuals (3.3%) and male snails mounting females' shells (6.7%). Animals adjacent to eggs, adjacent to egg layers, or in the act of depositing egg capsules on the substratum comprised 15.8%, 19.2%, and 20.8%, respectively, of the snails encountered.

A potential bioassay to test egg-laying aggregations for pheromone production is being investigated. Individual snails from study sites with aggregations were acclimatized in a sample jar containing 60 ml of filtered sea water. One ml of control (filtered sea water) or stimulus (water obtained adjacent to an egg-laying aggregation) was pipetted approximately 1 cm anterior of each oyster drill shell and the behavior of the snail monitored for 1 minute. Response was considered positive if cephalic antennal extension was observed.

	Response	No Response	$\Sigma$
Stimulus	26	22	48
Control	3	45	48
$G_{adj} = 26.20^{***}$ $X^2_{(0.005)} = 7.88$ d.f. = 1			

Independence of the response to the stimulus was tested with the G-test using Yates' correction. The null hypothesis of response being dependent on the stimulus is statistically significant at the 0.005 level in these preliminary experiments. More work needs to be done to test the specificity of the response to different snail types and stimuli, and to document the role that cephalic antennal elongation plays in oyster drill aggregation behavior.

# AN ORTHONECTID PARASITE IN A NEW HOST, *MYTILUS EDULIS*, AND ITS RELATIONSHIP TO SEASONAL MUSSEL MORTALITIES IN SOUTH PUGET SOUND, WASHINGTON

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Experimental raft culture of the blue mussel *Mytilus edulis* was initiated in 1979 at the Squaxin Island Indian Tribe seafarm in south Puget Sound, Washington. In May 1979, a natural mussel set was collected on net panels. By November 1979, approximately 530 mussels, averaging 2.3 cm, were transferred to each of five 5-m-long Netlon bags. Growth was monitored by monthly measurements of mussel length and bag weight after removing fouling organisms. Survival and growth began to decline in June 1980 and by November only 12% (318 out of 2,650) of the experimental population had survived.

In the second attempt, 60 bags with approximately 400 mussels in each were suspended from rafts in November 1980. To assay for mortality due to predation, half of the population was protected by a predator net. Mussels collected in May 1980 reached market size ( $\bar{X} = 5.6$  cm) within a year, but by August 1981 cumulative mortality exceeded 81% in protected and 57% in unprotected mussels. The netting apparently reduced predation because the unprotected raft had 23% fewer mussels than did the protected raft. Although predators undoubtedly contributed to the decline in numbers of *M. edulis*, the majority of "summer mussel mortalities" were unexplainable. (Summer mortalities of *M. edulis* have also been observed by others in Budd Inlet and other areas of south Puget Sound.)

In 1981, in an attempt to explain the causes of these mortalities, mussels were sent to the University of Washington, School of Fisheries, for pathological evaluation. Both histological sections and fresh squashes of *M. edulis* tissue were examined. Moderate-to-heavy infections of an orthonectic mesozoan of the genus *Stoecharthrum* were present in 15% of 73 mussels examined from 1 to 30 July 1981. This parasite is not only different from the only described species of *Stoecharthrum* (*S. gardi* Caullery and Mesnil), but is also a new host record for the genus and the first report of an orthonectid in a North American mollusc. The parasitized mussels, 5.8 to 6.8 cm long, often contained a reduced number of gametes and frequently appeared to be emaciated. In moderate infections, the orthonectids

were usually restricted to the mantle and gonads, but in heavy infections the parasites were also present in the digestive gland, adductor muscle, and ctenidia. Some of the mussels were effectively castrated, and about half were moribund with gaping valves.

Additional field studies are necessary to determine the incidence and range of *Stoecharthrum* in *M. edulis*, and to assess the relationship between these parasites and the observed mortality. Other factors which may contribute, either singly or synergistically, to mussel mortalities include interspecific competition with fouling organisms, intra-specific competition within and between mussel strings, post-spawning reduction in glycogen levels, environmental toxicants, and adverse water conditions (e.g., toxic plankton blooms and metabolites, extreme water temperature, salinity, pH, and oxygen levels).

#### STUDIES ON THE ORIGIN AND NATURE OF TOXICITY IN ALASKAN BIVALVES: TOXINS FROM *PROTOGONYAULAX* OF THE NORTHEAST PACIFIC

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In an effort to understand the source and nature of toxicity in Alaskan shellfish, we isolated dinoflagellates from several locations along the Pacific coast, and studied the toxin composition and net toxicity of those cultures. Although field studies have not been accomplished that would determine the significance of our findings outside the laboratory, our results have the following implications which may be important to the shellfish industry.

1. All toxic isolates obtained so far fall within the genus *Protogonyaulax*—the group that has generally been thought responsible for paralytic shellfish poisoning (PSP). Although the status of the individual species (*P. catenella*, *P. tamarensis*) is dubious, the established view that *Protogonyaulax* is responsible for toxicity seems on the right track.

2. The resting cysts of *Protogonyaulax*, which look quite different from motile cells, were found in the sediments from most locations sampled, from San Francisco to the Aleutian Islands. Their abundance varies widely. At some locations the concentration may be sufficient such that the cysts themselves could be an important source of toxicity.

3. The toxicity per cell varies significantly with growth conditions, being several times higher under conditions likely to be found in nature than under those conditions that past studies have used for growth in the laboratory. These levels can be sufficiently high (over 2,000 µg PSP per ml of packed cells) so that significant levels of PSP in marketed products could be caused by undigested cells contained in the shellfish.

4. Six new toxins have been isolated from our cultures, each of which can be converted to one of the six previously known toxins under mild conditions. This conversion increases the toxicity by a factor that, depending on the toxin, ranges from 6X to over 70X. The conditions under which this conversion occurs are such that it may happen during processing, cooking, or consumption of the product, but may not be complete under the conditions used for sample preparation in the standard AOAC (Association of Official Analytical Chemists) mouse bioassay. If these new toxins occur in shellfish, it is possible, therefore, that the mouse assay, as currently prescribed, does not fully assess risk to the consumer.

5. Each *Protogonyaulax* isolate produces some, but not all, of the 12 toxins. In contrast to net toxicity, which varies with growth conditions, toxin composition appears to be a relatively stable characteristic of an isolate. Composition, however, varies with location; all isolates from the same location have the same toxin composition while those from different regions are quite distinct. To date, we have identified six different regions, each with its characteristic composition, varying from one that produces only saxitoxin with a trace of neosaxitoxin, to one that produces nine toxins of the twelve possible. Because the toxins differ in their potency, stability, and binding properties, the nature of the toxin being supplied by the dinoflagellates to the shellfish and, therefore, the nature of shellfish toxicity, will vary from one location to another but will be constant within each region.

#### PRACTICAL METHODS OF HANDLING AND SETTING EYED-PEDIVELIGER LARVAE OF THE PACIFIC OYSTER *CRASSOSTREA GIGAS* (THURNBERG)

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Hatchery production of eyed-pediveliger oyster larvae is capable of supplying the commercial oyster industry with a consistently uniform, regionally available source of quality oyster seed.

In handling hatchery-reared larvae, either during shipment or in storage at the commercial growers setting facility, they can be held out of their normal aqueous environment, maintained at 5°C in a moist wrapping for up to 6 days without noticeable depreciation in health, setting, or juvenile growth abilities.

In setting experiments, maximum larval set occurred at temperatures between 25 to 30°C, with no larval settlement at 40°C. No interaction with temperature over a range of salinities (15 to 35 ppt) could be shown.

The possibility of chemotactic inducement of larval settlement and metamorphosis with selected concentrations of related catecholamines is currently being examined. Specifically, L-3,4-dihydroxyphenylalanine (L-DOPA) in solution at  $10^{-6}$  M (1.972 ppm) can induce competent eyed-pediveliger larvae of *Crassostrea gigas* to settle and metamorphose shortly after exposure under proper conditions.

#### PUGET SOUND MUSSEL STUDIES

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Mussels have been an important food item in Europe for years, but only in the past decade have they begun to be accepted as an important food item on the west coast of the United States. The University of Washington with the support of the Washington State Department of Fisheries and the National Office of Sea Grant has performed research to help this industry grow. A summary of the results follows.

Mussels from different populations in Puget Sound which were cultured at the same location differed in growth and survival rates.

Penn Cove seed grew to a greater length in 4 months than did Dabob Bay seed, and Dabob Bay seed outgrew Budd Inlet seed at each of three locations in Puget Sound.

The differences in growth and survival of mussel populations when held in similar environments suggest that genetic differences may exist between mussel populations in Puget Sound.

Growth of *Mytilus edulis* from Budd Inlet seed showed similar high growth rates at Penn Cove, Dabob Bay, Case Inlet, and Budd Inlet. Manchester seed had slower growth rates than the others. All of these groups experienced substantial mortality rates of up to 85% mortality.

*Mytilus californianus* initially grows slower than *M. edulis* but seems to grow well once established. *Mytilus californianus* also does not seem to exhibit the mass mortalities that *M. edulis* does in a side-by-side culture test.

Production or biomass of *M. californianus* initially was slow but after 6 months it exceeded the biomass production of *M. edulis*.

*Mytilus californianus* might be cultured in areas where *M. edulis* cannot because of mortality problems.

#### DYNAMICS OF A TOXIC DINOFLAGELLATE BLOOM

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Outbreaks of paralytic shellfish poisoning (PSP) that required harvesting closures have become more frequent, lasted longer, and have covered larger areas in Washington in the past several years.

A routine sampling program to study changes in the abundance of *Gonyaulax catenella* Whedon and Kofoid, the causative organism of PSP, associated environmental conditions, and PSP levels in shellfish has been carried out in central Puget Sound. The information will contribute to the development of a predictive method and, possibly, eventual control of the problem under specialized conditions. A bloom occurred in one of the study areas in June 1981, and provided an opportunity to study the hydrography, growth dynamics, diel migration, and biological interactions.

Preliminary observations of samples and data from the bloom period indicate that factors associated with the buildup in cell numbers were increases in both temperature and intensity of stratification. During the 3 days prior to the peak of the bloom, the growth rate of *G. catenella* was one division per day. The sharp decline in cell density was associated with three factors: (1) a marked reduction in concentrations of  $\text{PO}_4$  and  $\text{NO}_3 + \text{NO}_2$ , (2) diminished stratification, and (3) a dramatic rise in the proportion of the population parasitized by an endoparasite, *Amoebophrya ceratii*.

A study of division rates of *G. catenella* under field conditions by the paired nuclei technique suggested that, because of the horseshoe shape of the nuclei of this species, a better technique is counting the small daughter cells that have globular nuclei.

Both drogue and transect studies to determine whether *G. catenella* undergoes diel vertical migration indicated that this organism migrated to depths of 6 to 8 m by 2200 hours and initiated the upward migration before dawn. The rate of migration was approximately 0.5 m/hr. The fact that *G. catenella* does undergo diel migration has implications for its horizontal as well as vertical distribution and, under conditions of this bloom, for the availability of nutrients.

# **SHELL GROWTH AND CARBOHYDRATE CONTENT IN THE PACIFIC OYSTER *CRASSOSTREA GIGAS* (THURNBERG) DURING GAMETOGENESIS**

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The reproductive cycle dominates the life of the Pacific oyster *Crassostrea gigas* (Thurnberg). Extensive gonad proliferation occurs in environments characterized by warm temperatures (20°C and higher) and high productivity. Gametes occupy as much as 40% of the dry tissue weight of 2+-year-old animals.

Although many bivalves partition energy reserves to different areas (e.g., shell deposition, gonadal tissue, somatic tissue, etc.) during gametogenesis, the Pacific oyster appears to direct an extensive amount of energy toward the reproductive cycle. In many bivalves, shell growth occurs seasonally during periods of increased temperature and seston. Typically, shell growth occurs steadily over this period uninfluenced by reproductive activities. This has been shown in the eastern oyster *Crassostrea virginica*, and the European flat oyster *Ostrea edulis*. Shell growth in those species is normally correlated to changes in temperature and ration.

The carbohydrate cycle in the Pacific oyster is similar to that of many bivalves in that stored reserves are depleted during gametogenesis and then stored once again following spawning and/or resorption. The cycle in the Pacific oyster is correlated very precisely to activities occurring in the gonad.

The influence of the gametogenic cycle on both shell growth and the carbohydrate cycle was demonstrated in two 1-year-old full-sib families replicated in two bays in southern Puget Sound during the summer of 1980. Two distinct patterns of gametogenesis were observed between the two bays. In one bay (Mud Bay), the typical pattern of development mentioned earlier occurred with extensive gonad still present (although undergoing resorption) in early November. In the second bay (Oakland Bay), however, replicates of the same experimental groups, which spawned in July, quickly repopulated and spawned again within 2 weeks. Resorption of residual follicles followed and, by mid-September, most animals were undifferentiated.

Shell growth and glycogen levels differed between the two bays and appeared to be entrained by gametogenic activities. No shell growth occurred during gonadal proliferation in May, June, and July, but did occur following spawning (in Oakland Bay) or peak development (in Mud Bay). Glycogen storage occurred after the second spawning in Oakland Bay (in August–September), and after resorption was well underway in Mud Bay (October).

Changes in environmental parameters were very similar in the two bays. Temperature, salinity, and organic and inorganic particulates showed similar trends in the two bays.

The data suggest that the dynamics of shell growth and carbohydrate content are entrained more by the gametogenic cycle than by environmental parameters such as temperature or seston in oysters that exhibit extensive gonadal development in environments characterized by high nutrients and warm temperatures (> 20°C).

# **FEEDING HABITS OF THE DUNGENESS CRAB *CANCER MAGISTER* DANA IN GRAYS HARBOR, WASHINGTON**

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As part of a study of the distribution and ecology of *Cancer magister* Dana in Grays Harbor, Washington, we examined the contents of 410 stomachs from crabs collected at various sites and in various combinations of tide and light conditions. Prey items were identified, counted, and their weight estimated as a percent of total contents dry weight. An index of relative importance (IRI) was calculated for each prey taxon in a sample by the equation:

$$\text{IRI} = \% \text{ frequency of occurrence} \times (\% \text{ of total prey numbers} + \% \text{ of total prey weights})$$

Use of different types of prey changed throughout a diel cycle (mean of four seasonal 24-hour collections; total = 341 crabs). Fish were the most important prey group, indicated by their high IRI, during day high tides. Although fish remained the most important food, bivalves increased in importance during day low tides. Use of crustaceans increased greatly at night, though most of this use occurred at one intertidal site where the IRI for *Crangon* shrimp increased 20-fold from day to night, reflecting a concurrent increase in shrimp abundance as indicated by trawl samples.

Small crabs (< 60 mm) were found to consume primarily small bivalves and small crustaceans, but few fish or *Crangon*. Bivalve use decreased among older age groups. *Crangon* spp. were preyed on most heavily by medium-size crabs (60–100 mm) and less by large crabs (> 100 mm). Fish were used greatly by medium-size crabs, but were the primary food item of large crabs in Grays Harbor. Fish species eaten included sand lance (*Ammodytes* sp.), lingcod (*Ophiodon* sp.), longfin smelt (*Spirinchus* sp.), and tomcod (*Microgadus proximus*). Cannibalism occurred by all age groups in the order small > large > medium-size crabs.

# GROWTH AND DECLINE OF *GONYAULAX CANTENELLA* BLOOM ASSOCIATED WITH PARASITISM

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During examination of *in situ*-growth rates of a bloom of the flagellate *Gonyaulax catenella* Whedon and Kofoid, which causes paralytic shellfish poisoning (PSP), we noted a dramatic rise in infestation by the dinophycean parasite *Amoebophrya ceratii*. This parasite has previously been recorded in wild populations from Washington, but this is the first series of observations implicating it as an important natural antagonist to *G. catenella*. The observations are significant because they suggest a possible biological control agent.

Examination of formalin-fixed, acetocarmine-stained plankton samples revealed that peak abundance of the host was followed by a rapid increase in the proportion parasitized. The parasite increased from 2% infestation at the peak of abundance and shellfish toxicity to nearly 50% in 5 days. This proportion continued until bloom termination. During the period of increasing parasitism, the rate of infestation was about 2.5 times as great as the maximum observed *G. catenella* growth rate; therefore, the parasite is probably a major contributor to the decline in the bloom. The high infestation rate is due in part to the ability of the parasite (reported here for the first time) to invade one cell of the chain-like host and remove nuclear material from adjacent cells. Thus, *G. catenella* appears to be highly advantageous to pervade, and low-host densities are concomitant with high proportions of parasitism. Initial stocks of the parasite may have come from *Peridinium trochoideum*, which is also infested.

The diel vertical patterns of parasitism resemble those of migrating cells, and the parasitized chains were observed to retain their motility. Observations on relative numbers of stages in the course of the infestation show only minor changes during the bloom. This suggests that the life cycle of the parasite is rapid.

The rapid response, apparent host preference, and potential for growing the parasite in a nontoxic host for dispersal indicate that *A. ceratii* could serve as a biological control agent for PSP.

# THE INCIDENCE AND SEASONAL DISTRIBUTION OF *YERSINIA ENTEROCOLITICA* AND *VIBRIO* *PARAHAEMOLYTICUS* IN A PUGET SOUND COMMERCIAL OYSTER BED

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In the past few years there has been increasing interest in lesser known bacteria in the genus *Vibrio* and the genus *Yersinia*. These genera have been associated with food-borne illness outbreaks. The U.S. Food and Drug Administration has sponsored research to understand more about these organisms which have been associated with marine food products.

The research project was designed to simultaneously seek information on *Yersinia* and *Vibrio* in Puget Sound. The goals of the project were: (1) to assess the workability of methodology developed for food samples for the analysis of environmental samples; (2) to study the presence, levels, and seasonal distribution of *Yersinia* and *Vibrio* in a commercial oyster bed; (3) to determine the relationship of *Yersinia* isolated from these environmental sources to those which have caused disease in man; (4) to look at the incidence of *Vibrio vulnificus* and *V. cholerae*; and (5) to evaluate the relationship of *V. parahaemolyticus* to *V. alginolyticus*.

Assisted by the Pacific Coast Oyster Growers Association, Rocky Bay on Case Inlet was chosen as the sampling site. This commercial oyster bed typified conditions of commercial oyster beds in Puget Sound. The project was designed to include five 7-day sampling trips spaced throughout the year. A total of 227 bay water, 210 sediment, 227 oyster, and 34 creek water samples was collected and analyzed throughout the year. *Yersinia enterocolitica* was present in relatively high numbers in the creek water throughout the year. This indicates the creek as an important source of *Yersinia* in the bay itself and shows that *Yersinia* levels are not related to the sporadic fecal coliform levels. In the bay water and oyster samples, the sharp seasonal peak in the numbers of *V. parahaemolyticus* is not well associated with numbers of *V. alginolyticus* or fecal coliforms. *Yersinia enterocolitica* predominated in the colder months. Japanese researchers have proposed using the level of *V. alginolyticus* as a market standard for safety of oysters from *V. parahaemolyticus*.

Comparing the data from *V. parahaemolyticus* for all sample types, sharp peaks occur in August for levels in bay water and oysters in contrast to a broader level for

sediment which is due to attachment to algae in the mud as temperatures drop. However, average levels of *V. alginolyticus* did not correlate well with *V. parahaemolyticus* and were often exceeded by the latter. No *V. cholerae* or *V. vulnificus* were isolated from Rocky Bay.

The *Yersinia* pattern is reversed with peak values occurring in the winter months. Highest levels were obtained in February. Isolates of *Y. enterocolitica* were classified under three modified systems devised by Schieman and were found to fall into 19 different biotype patterns, indicating tremendous diversity within the population sampled. Thirteen serotypes were identified of 100 isolates tested, including 4 serotypes often associated with human diseases. Many produced enterotoxin; many were lethal for mice. None of 11 isolates tested was entero-invasive. This again shows the diversity of the population.

The levels of *Y. enterocolitica* and *V. parahaemolyticus* are not sufficient to indicate a clear hazard from the consumption of these raw oysters, but are significant and should not be ignored.

#### CHEMOTACTIC ORIENTATION TO PREY BY THE ATLANTIC OYSTER DRILLS *UROSALPINX CINEREA* (SAY)

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The eastern oyster drill *Urosalpinx cinerea* (Say) is a shell-boring snail that preys upon numerous species of sessile, shelled, and encrusting invertebrates, many of which are commercially valuable. Newly hatched, nascent snails were used to develop a powerful bioassay for chemotactic orientation to prey. An activity chamber constructed of 2-ml pipets was used to assay rheotactically facilitated chemotaxis of nascent snails to small amounts of stimulus (0.05–5.0 ml) diluted to 2.0–80.0 ml in filtered seawater. The assay design required upstream locomotion of at least 1 cm within 10 minutes to record a positive response.

Specificity of chemotaxis was tested by assaying the response to 25 species of marine invertebrates and fish. Only balanoid barnacles and a mixture of two bryozoan

species produced an effluent that was highly attractive to nascent snails. Oysters (*Crassostrea virginica*) produced an effluent that was only weakly attractive, evoking at most a 20% response. The mussel *Mytilus edulis*, commonly preyed upon in nature, did not evoke a significant response.

Laboratory experiments were performed to determine the manner in which nascent snails behaviorally integrate competing chemical cues emanating from co-occurring species of prey. Mussel stimulus inhibits chemotaxis to barnacles, although it does not evoke chemotaxis by itself. Oyster stimulus inhibits snail chemotaxis to high concentrations of barnacle stimulus, but facilitates that to low ones. Continuous 2-hour exposure of nascent snails to either barnacle, mussel, or oyster stimuli causes a diminution of chemotaxis upon subsequent exposure to barnacle stimulus, suggesting that inhibition or facilitation of chemotaxis to stimulus mixtures are not caused by one stimulus masking a second while free in seawater. We infer that snails perceptually or behaviorally integrate chemical information in barnacle-oyster and barnacle-mussel mixtures of stimulus water.

We next tested the hypothesis that *U. cinerea* could become "conditioned" or sensitized to the odor of prey other than barnacles. Survivorship, growth, and rapacity were measured for newly hatched and 1- to 7-week-old snails maintained on single species diets of either mussels, oysters, or barnacles. Snails that were fed barnacles grew the most, survived the best, and consumed more prey than those fed either mussels or oysters. At the end of the 7-week conditioning period, oyster-fed snails were less sensitive to barnacle stimulus and more sensitive to oyster stimulus than either barnacle-fed or nascent snails. At high-stimulus concentrations, oyster-fed snails responded about equally to oyster and barnacle stimuli. Barnacle-fed snails were less sensitive to oyster stimulus than either oyster-fed or newly hatched snails. Mussel-fed snails survived too poorly to enable comparisons of chemotaxis with either oyster- or barnacle-fed snails. These results suggest that *U. cinerea* can become sensitized to the odor of other prey species without losing its innate chemotaxis to barnacle odor.

Isolation and chemical characterizations of barnacle stimulus by adsorption chromatography and high-pressure liquid chromatography techniques are presently under investigation.



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**COVER MICROPHOTOGRAPH:** The shell hinge region of a 2-day old larva of the Atlantic ribbed mussel *Geukensia demissa* (Dillwyn). Examination of hinge structures provides a means of unambiguously identifying bivalve larvae isolated from plankton samples (see page 65). The specimen depicted was immersed in a 5% solution of sodium hypochlorite for approximately 10 minutes to facilitate separation of shell valves. After rinsing in distilled water, the disarticulated shell was mounted on copper tape, coated (under vacuum) with approximately 400 Å of gold-palladium, and photographed under an ETEC Autoscan scanning electron microscope (magnification = 2100X). [Micrograph by R. A. Lutz and A. S. Pooley of Rutgers University and Peabody Museum, Yale University, respectively.]

# JOURNAL OF SHELLFISH RESEARCH

VOLUME 2, NUMBER 2

DECEMBER 1982



*The Journal of Shellfish Research* (formerly *Proceedings of the  
National Shellfisheries Association*) is the official publication  
of the National Shellfisheries Association

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*Journal of Shellfish Research*

Volume 2, Number 2  
ISSN: 00775711  
December 1982

This issue of the *Journal of Shellfish Research* is dedicated to the memory of  
**DR. LYLE STANHOPE ST. AMANT**

Marine Biological Laboratory  
APR 27 1984  
Woods Hole, Mass.



**DR. LYLE STANHOPE ST. AMANT**, Lyle or "Doc," as his many friends and associates knew him, was born 10 April 1915 in New Orleans, LA. He was educated at Louisiana State University (B.S., 1935; M.S., 1938) and Northwestern University (Ph.D., 1941). He served in the U.S. Navy Medical Corps during World War II, and began his 34-year career with the Louisiana Department of Wildlife and Fisheries 1 March 1946 as a wildlife biologist. On 26 January 1955, Dr. St. Amant was transferred to the Department's Oyster, Water Bottoms, and Seafood Division, where he subsequently became Division Chief in 1962. On 4 May 1969, he became the Chief Marine Biologist and the Director of the Louisiana Marine Research Laboratory on Grand Terre Island. Dr. St. Amant became the Assistant Director of the Department of Wildlife and Fisheries in 1971, with marine resources and their management as his specialty. Finally, on 1 October 1976, following reorganization of state government, he became Assistant Secretary and headed the Department's Office of Coastal and Marine Resources; he continued in that position until retirement on 30 June 1980.

During his 34-year fisheries career, Dr. St. Amant

authored more than 80 technical publications in the fields of marine and estuarine fisheries, ecology, and hydrocarbon pollution and development. He served on numerous commissions, committees, and advisory councils that dealt with marine and estuarine fisheries including the National Research Council, the U.S. Department of the Interior's Coastal and Estuarine Management Advisory Committee and Marine Affairs Action Group, the U.S. Department of Commerce's Estuarine Industrial Problems, National Sea Grant Advisory and Coastal Zone Management committees, the Federal Power Commission's Supply-Technical Advisory Committee of the Natural Gas Survey, the Gulf States Marine Fisheries Commission, the Gulf of Mexico Fishery Management Council (Chairman Emeritus), and the U.S. Congressional Office of Technology Assessment (as a consultant). Countless people at international, federal, and state levels sought his counsel and advice. He helped hundreds of organizations in reaching important decisions regarding management of valuable marine resources. Dr. St. Amant was recognized as a national and international authority on marine and estuarine matters including shrimp, crab, and oyster biology and management, and he was a champion of multiple use of natural resources including estuarine shellfish and the oil, natural gas, and sulfur deposits that were below coastal estuaries.

Dr. St. Amant received numerous distinguished awards, certificates, and plaques from various local, state, and national groups including the Governor's Award as the outstanding professional in fisheries conservation in 1964 (presented by the Louisiana Wildlife Federation), the Conservationist of the Year award in 1970 (by the Louisiana Outdoor Writers Association), and the selection as Honorary Member of the National Shellfisheries Association in June 1973. In his honor, the Louisiana Wildlife and Fisheries Commission renamed the Grand Terre Marine Research Laboratory the "Dr. Lyle S. St. Amant Marine Laboratory," certainly a fine tribute to his professional life and accomplishments.

**DR. LYLE STANHOPE ST. AMANT** passed away on 21 December 1981 at the age of 66. He is survived by his wife Monroe, of Hammond, LA; two sons, Joseph and William; a daughter, Katherine; and hundreds of friends and colleagues in Louisiana and the NSA. Shellfisheries of the Gulf of Mexico have benefitted from his work and management efforts; he will be missed, but his legacy remains.

Ronald J. Dugas and McFadden Duffy  
(Louisiana Department of Wildlife and Fisheries)



## ANAEROBIC MORTALITIES OF OYSTERS IN VIRGINIA CAUSED BY LOW SALINITIES

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**ABSTRACT** Oysters on natural beds in the upper seed area of the James River died anaerobically in the winter and early spring of 1979-80 during prolonged exposure to fresh water and low salinities ( $< 5$  ppt). Heavy rains in the fall of 1979 combined with the usual winter-spring runoff to produce low salinities. Oysters in trays were transplanted in late March and early April to six high-salinity areas where mortalities were found a month later. The oysters died slowly within closed shells because they were unable to feed and respire in the nearly fresh water. This produced a strong, malodorous stench and blackened shell margins that are characteristic of anaerobic decay. Similar phenomena occurred previously in the Rappahannock River about 1 May during several wet years during the past three decades. At depths of 5 to 6 m, dissolved oxygen was depleted and everything on the bottom became black with iron and other heavy metal sulfides. Dead oysters were not discovered until June after waters had become aerobic again.

### INTRODUCTION

For 21 consecutive years, disease-free oysters from low-salinity waters of the James River were transplanted to high-salinity waters in several rivers of Virginia to monitor the incidence of "Delaware Bay Disease" (or MSX) which is caused by the haplosporidan *Minchinia nelsoni* Haskin, Stauber, and Mackin (1966). This pathogen requires water salinities of  $\geq 15$  ppt to attack oysters effectively. Horsehead Rock in the upper seed area of the James River (Figure 1) has annual salinity maxima of 12 to 15 ppt in late summer; its oysters are usually free of diseases and parasites including *M. nelsoni*. Because salinities are low in the James River seed area, little selection by MSX has occurred since its introduction to Chesapeake Bay in 1959 (Andrews and Wood 1967). These oysters are, therefore, relatively susceptible to the disease and are used for comparisons of annual intensities of MSX infections and mortalities in Chesapeake and Delaware bays (Haskin and Ford 1982).

During late winter and spring, Horsehead Rock oysters are routinely exposed to low-salinity and even fresh water which causes suspension of normal feeding activities, and aerobic respiration is interrupted for months. Oysters usually withstand this low-salinity stress during cold water temperatures by closing their valves and becoming dormant or narcotized (Andrews et al. 1959). In years of heavy rainfall and runoff, this state of anaerobiosis may extend to 1 May without serious oyster mortalities, provided that the dormancy is not interrupted by a period of normal feeding activities. Oysters begin to filter feed when water salinity and temperature approach 5 ppt and  $10^{\circ}\text{C}$ , respectively.

Fall and winter of 1979-80 were wet in Virginia because of record rainfalls in September and November 1979. Oyster spatfall in the James River in September 1979 was light in intensity, but useful in the post-MSX years after 1959 when only light or insignificant spatfalls occurred. Most small spat ( $< 5$  mm) located from Wreck Shoal to Horsehead Rock

were killed in late fall by low-salinity water. Larger and older oysters including yearlings survived during the fall and winter except at Deep Water Shoal, the upper river seed bed exposed to the most fresh water. Few boxes were found when Horsehead Rock oysters were dredged in March for experimental tray studies of MSX. Approximately one third of the oysters at Horsehead Rock died in the spring of 1980 as low salinities persisted, but all oysters died at Deep Water Shoal.

### MATERIALS AND METHODS

Experimental oysters were usually collected in March before their dormancy period ended to avoid inclusion of dying oysters (gapers) and hidden boxes (empty shells). This prevented occurrence of dead oysters in trays for a month or two after transplantation into high-salinity waters. Apparently, some oysters died with tightly closed shells in March 1980, before transplantation, and slow anaerobic decomposition was initiated in cold waters ( $< 5^{\circ}\text{C}$ ). The oysters were held at the Virginia Institute of Marine Science (VIMS) pier for short periods while being sorted and counted. This allowed them to adjust to moderate salinities before transplantation to higher salinities. The trays of oysters were then distributed to stations in three rivers along the Western Shore of Chesapeake Bay and on the seaside of Eastern Shore for disease monitoring. Shells of dead oysters did not open and deaths were not discovered during handling operations.

Trays of oysters were placed on natural beds to monitor diseases and mortalities without the influence of predation and adverse siltation (Andrews et al. 1962). Each tray held  $0.036 \text{ m}^3$  (1 bu) of oysters, was completely enclosed with 2.5-cm (1-in.) galvanized mesh hardware cloth, and was raised 0.3 m above the bottom on legs. Monthly examinations were made to determine the number of live and dead oysters, to collect gapers for disease testing, and to remove

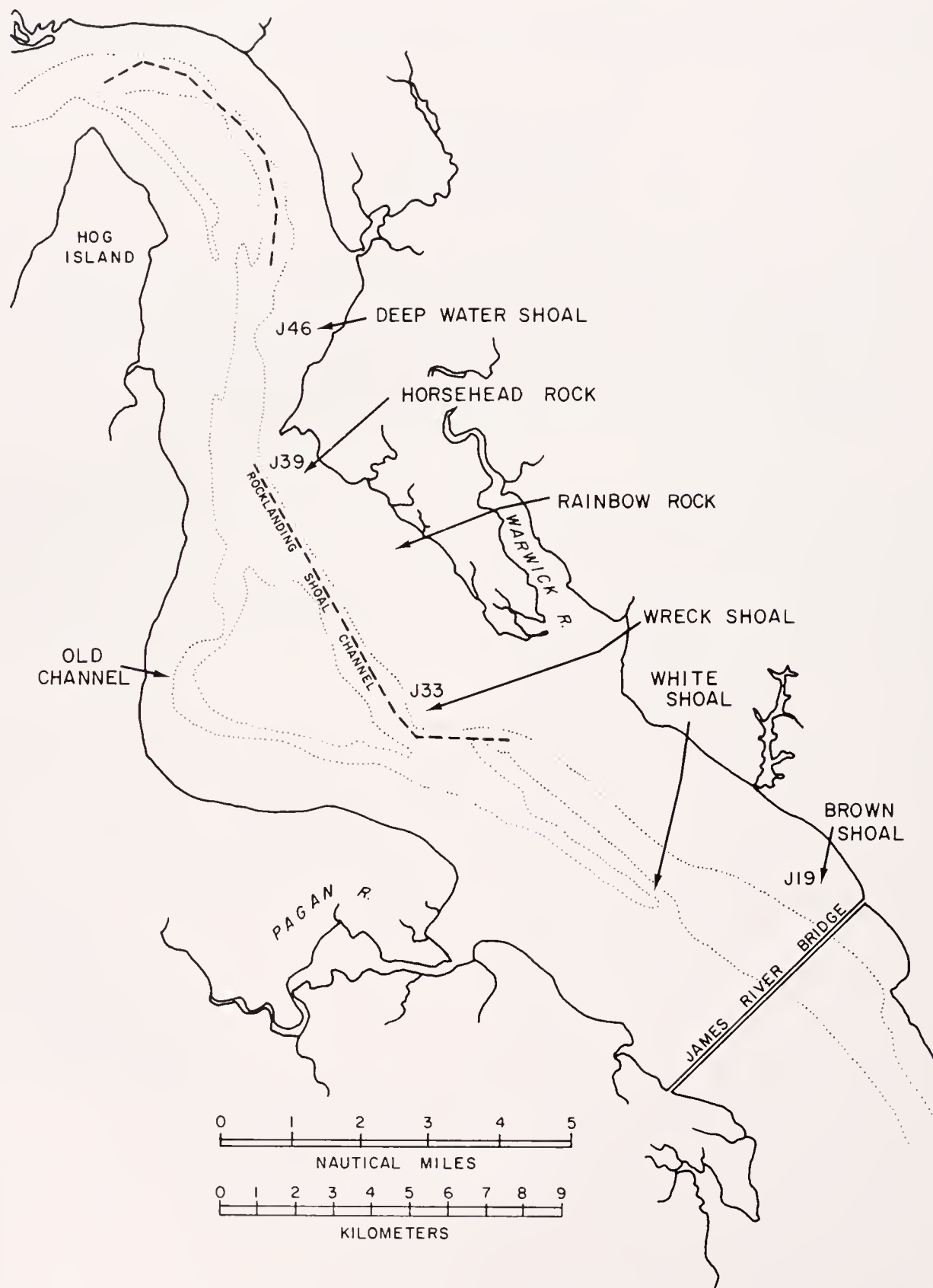


Figure 1. Map of seed-oyster area in James River, Virginia. Natural oyster beds extend from the bridge (J19) to Deep Water Shoal (J46). (Distances are expressed in kilometers from the river mouth.)

fouling organisms. This method of monitoring disease has been used for 25 years in Virginia, particularly in areas where planting of oysters has ceased because of MSX mortalities. Oysters were handled individually and randomized in the trays during examination.

### RESULTS

The first lots of oysters were dredged from Horsehead Rock and transplanted to the VIMS pier in the York River on 20 March 1980. No evidence of dead or dying oysters was observed at that time. On 26 March, the oysters were sorted again for boxes, and three trays with 500 oysters each were moved to a Gloucester Point station above the York River Bridge for monitoring disease prevalences and mortalities. These tray oysters were re-examined on 23 April and the mortality was  $< 1\%$  for the 28-day period (8 of 1,500 dead). The next examination on 13 May revealed many boxes and dead oysters with slimy meats. The deaths were unusual because the shells remained closed after the tissues

became soupy. The oysters did not exhibit the usual hollow sound characteristic of "cluckers" or empty shells. Dead oysters were recognized by black anaerobic streaks along the shell margin or bill, and they exuded an extremely malodorous and sulfurous stench that is typical of anaerobic decomposition.

By 6 June 1980, most dead oysters had been detected and the survivors had new shell growth. The mortality was almost identical in the three trays at Gloucester Point for a mean of 27.5% (412 of 1,500 dead) (Figure 2). Oysters in trays that were moved to James River, Rappahannock River, and Mobjack Bay showed similar timing and extent of mortalities (Table 1). Boxes and gapers began appearing about one month after transplantation to high-salinity waters. Occurrence of boxes ceased after about 1 June at Gloucester Point (Figure 2) although some trays in other rivers were not examined until later. Surviving oysters appeared healthy with sharp new shell margins. Typical MSX mortalities began about 1 August 1980.

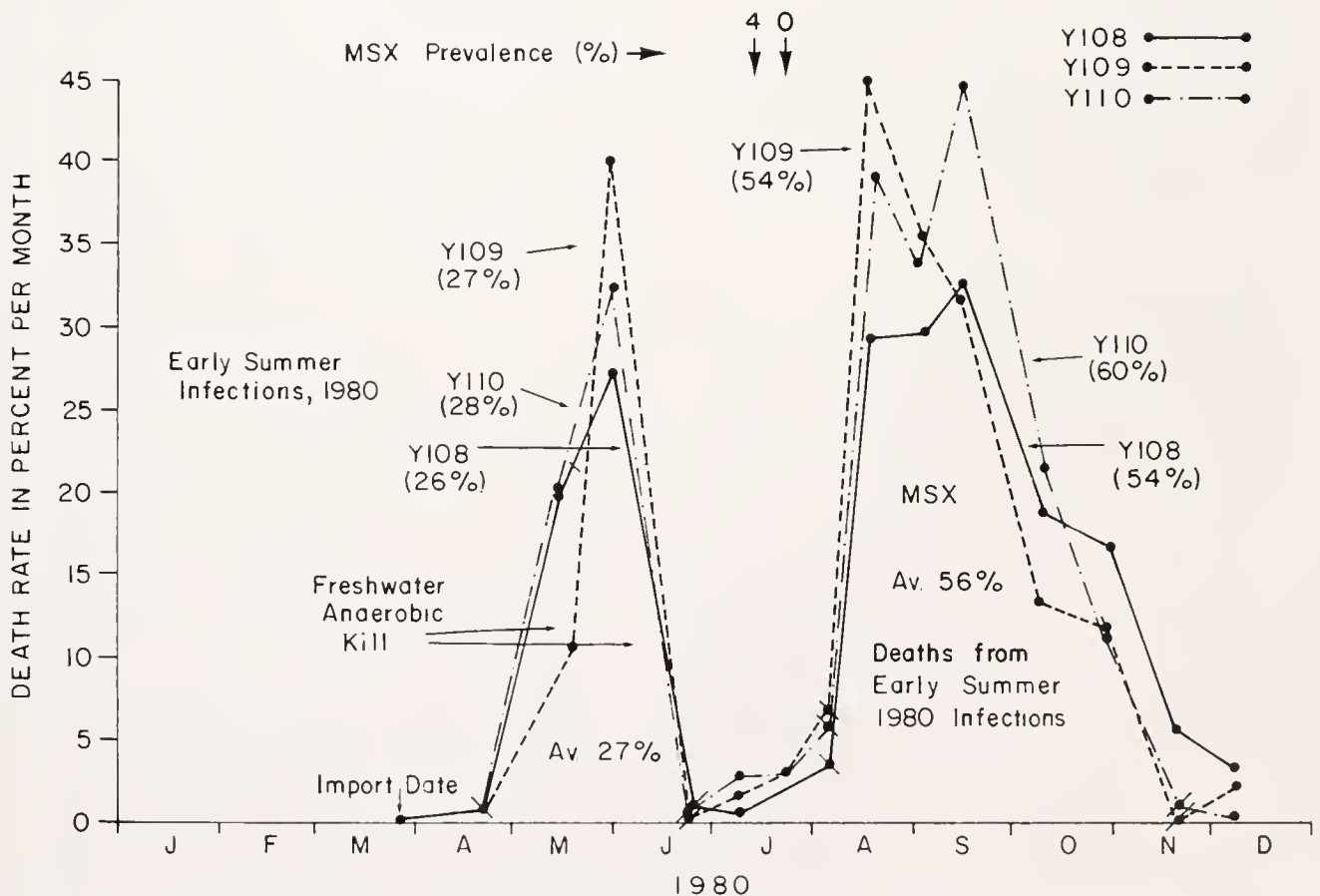


Figure 2. Mortalities from anaerobic deaths in May and from *Minchinia nelsoni* (MSX) in late summer and fall are shown for three replicate lots of oysters held in trays at Gloucester Point, Virginia. Two samples of 25 live oysters each were taken in July, too early to show intensity of MSX infections; 30 of 37 gapers (81%) had the disease after 1 August. Almost a month elapsed before anaerobic deaths became apparent. No further deaths occurred after 29 May when peak mortality rates occurred in all trays.

TABLE 1.  
Anaerobic mortalities of Horsehead Rock oysters  
transplanted in early spring 1980 to  
higher salinity waters.

Location	Tray	Transplant Date	Mortalities		
			Dates <sup>1</sup>		Total (%)
			Begun	Ended	
York River	Y108	20 Mar	23 Apr	29 May	26
(Gloucester	Y109	20 Mar	22 Apr	29 May	27
Point)	Y110	20 Mar	24 Apr	29 May	28
Mobjack Bay	MJ31	20 Mar	25 Apr	16 Jun	37
Piankatank River	PK16	2 Apr	5 May	7 Jul	30
Rappahannock River					
Parrotts Rock	R42	2 Apr	5 May	7 Jul	31
Balls Point	R43	2 Apr	5 May	7 Jul	32
James River					
Hampton Bar	J52	8 Apr	29 Apr	28 May	43
Brown Shoal	J53	8 Apr	29 Apr	19 Jun	45
Wreck Shoal	J54	8 Apr	29 Apr	19 Jun	43
Seaside of Eastern Shore Chicoteague					
Bay	S126	20 Mar	30 Apr	2 Jun	33
Swash Bay	S129	20 Mar	1 May	3 Jun	33

<sup>1</sup>Refinement of periods of mortality was limited by dates of examination of trays which were usually 3 to 5 weeks apart. The Gloucester Point data were best defined with counts on 13 May, 20 May, and 6 June, in addition to dates listed in table.

## DISCUSSION

Oysters that were transplanted as late as 8 April 1980 showed no external signs of anaerobic mortality. The discovery of deaths depended on timing of examinations; therefore, dates in Table 1 are not precise for the duration of anaerobiosis or the time of death. Late transplantations, however, resulted in higher mortality rates. This implies that the deaths were still occurring at Horsehead Rock although they were undetected because the shells were tightly closed.

The observed mortalities resulted from unusual weather and salinity regimes. The oysters were under low-salinity stress throughout 1979 from > 150 cm of rainfall over Virginia (compared to a 112-cm annual mean). The heavy rainfall and runoff were exceptional for the fall, and salinities were unusually low throughout the James River seed area (Tables 2 and 3). Additional data on mean salinities in the seed area for 12 years are given in Andrews and Hewatt (1957). Oysters on shallow (< 3 m) seed beds in the James River live in salinities close to those found at the surface in adjacent channels. An alert was issued by VIMS to warn of possible oyster mortalities from the low-salinity exposure. No mortality of adult oysters was observed in the fall and winter of 1979, and oystermen avoided the upriver seed

beds in the spring of 1980. Adult oysters, however, had very poor meats with Condition Indices of  $\leq 4.0$  (range = 4 to 15) (Hopkins, 1949).

$$CI = \frac{\text{dry weight of meat (g)}}{\text{volume shell cavity (ml)}} \times 100$$

TABLE 2.  
Typical surface salinities (ppt) in James River  
by seasons (means, 1952–1961).

Season	River Distance (km)		
	J0 (Mouth)	J33 (Wreck Shoal)	J46 (Deep Water Shoal)
Winter	19	8	0
Spring	16	11	0
Summer	20	16	10
Fall	22	16	5

TABLE 3.  
Surface and bottom salinities in upper half of James River  
seed area from October 1979 to June 1980<sup>1</sup>.

Date	Salinities (ppt) at Channel Stations, All Tides	
	Wreck Shoal (Nun "12" [J33])	Horsehead Rock (Nun "22" [J39])
20 Sep 79	10.7 [14.9]	5.0 [5.2]
26 Sep 79	1.2	0.1
2 Oct 79	1.9 [7.2]	—
10 Oct 79	1.5 [2.0]	—
15 Oct 79	1.7 [3.5]	1.4 [3.3]
22 Oct 79	6.0 —	—
24 Oct 79	9.2 [10.4]	3.4 [4.4]
30 Oct 79	7.4 [8.8]	3.4 [5.0]
10 Dec 79	1.9 [11.5]	1.4 [1.7]
17 Jan 80	10.6 [12.2]	6.2 [6.3]
28 Jan 80	4.7 [4.8]	0.9 [1.8]
14 Feb 80	13.2 [14.0]	8.4 [10.4]
19 Mar 80	7.9 [7.9]	0.9 [1.1]
10 Apr 80	3.3 [10.9]	0.5 [0.5]
23 Apr 80	1.5 [7.1]	—
2 May 80	6.3 [10.3]	1.9 [2.3]
9 Jun 80	6.9 [13.5]	6.0 [7.9]

<sup>1</sup>Bottom (12-m) salinities in brackets.

Oysters in the upper James River routinely enter winter dormancy about 15 December each year as water temperatures of > 5°C prevail. If salinities drop appreciably below 5 ppt, anaerobic dormancy occurs and water pumping for feeding and respiration is precluded. Closed oysters, even at warm temperatures, exhibit greatly reduced rates of heart beat and ciliary action (Stauber 1940); those oysters that are forced into anaerobic metabolism during winter exhibit no muscular or ciliary activity (Andrews et al. 1959). Winter-dormant oysters that were held at 20°C in fresh, well

water remained tightly closed, and internal salinity levels gradually declined over several weeks to months before they died (Andrews et al. 1959). The oysters at Horsehead Rock may have experienced intermittent closings and openings caused by the fluctuating salinities in late fall and winter of 1979–80. Anaerobic respiration is extremely wasteful of glycogen reserves in warm water, but metabolism is suppressed by low water temperatures in winter. Because rainfall continued to depress salinities through the winter, Horsehead Rock oysters were probably in a continuous state of anaerobic closure from about December 1979 through March 1980.

Oysters in winter dormancy normally respond quickly with increased cardiac and respiratory activity (heart beat and ciliary movement, respectively) when they are exposed to water of suitable salinity and temperature. In 1958, oysters that were opened in the laboratory, after months of winter dormancy in fresh water, began ciliary and cardiac activities in < 5 minutes after exposure to air (Andrews et al. 1959). When an oyster dies, the compressed hinge ligament usually opens the shell. When oysters died in the spring of 1980, their shells were held closed by the catch muscles, and the tissues decayed anaerobically. Presumably, those oysters were dead or moribund when transplanted to VIMS; they had lost their capacity to pump water by ciliary action of the gills. The consistent mortality rates in all 12 of the trays of transplanted Horsehead Rock oysters suggested that death or survival had already been determined before transplantation. On 8 April 1980, oysters for the last three trays were collected from Horsehead Rock and brought to Gloucester Point for acclimation to higher salinities. They were transplanted to stations in the James River on 29 April. Mortality was somewhat higher in those lots (43+%) suggesting that more oysters had reached the point of death during the extra 20 days in low-salinity water at Horsehead Rock.

The anaerobic deaths of oysters from prolonged exposure to fresh water caused abnormal sequences of death, with closed shells and a delay of about one month before their discovery. Similar occurrences of anaerobic deaths and delayed discovery were observed on deep water oyster beds in the Rappahannock River during periods of high freshwater flow. Those events, characterized as "black-bottom" phenomena, always occurred in late April or early May of wet years when spring freshwater discharge was highest. Dead oysters sometimes appeared later, after anaerobic conditions on the bottom had disappeared.

The black-bottom condition was observed in the Rappahannock River in at least four years (1949, 1953, 1958, and 1980). Freshwater flow rates and water temperatures at the Fredericksburg gauging station were well above normal during those wet winter and spring seasons (1 October to 1 May). In 1949, for example, the accumulative total for seven months of mean monthly flow rates for the period 1 October 1948 to 1 May 1949 was  $684 \text{ m}^3/\text{sec}$  (23,150 cfs),

whereas the 55-year mean was  $381 \text{ m}^3/\text{sec}$  (13,449 cfs). In addition, 10 cm of rain fell during the first 10 days of May over the watershed.

In 1949, the only year that mortalities were known to occur in the Rappahannock River, wide areas of public oyster grounds in 4 to 7 m of water became anaerobic in early May. Everything on the bottom, including oysters and mud, was blackened by iron and other heavy metal sulfides. The black color disappeared soon after the shells were exposed to air. Dredged materials had a strong, hydrogen sulfide odor. The oxygen deficiency was caused by a combination of the following factors which depleted the supply and/or prevented replenishment: (1) large accumulations of organic matter from heavy freshwater runoff; (2) intensive density stratification caused by warm fresh waters overlaying cooler, saline waters; (3) high oxygen demand created by rapidly increasing water temperatures in May; and (4) intensive phytoplankton blooms stimulated by high nutrient levels in the runoff. Because all of these factors existed, high oxygen demand near the bottom occurred when poor vertical mixing limited resupply. Mortalities were confined to deep oyster beds adjacent to the channel, whereas shallow beds had neither black bottoms nor oyster mortalities.

Earlier laboratory experiments documented anaerobic mortalities in oysters exposed to fresh water. Andrews et al. (1959) demonstrated that winter-dormant oysters maintained in fresh well water at  $20^\circ\text{C}$  died slowly with their shells closed and released malodorous sulfide gases.

Most oyster mortalities that result from exposure to low salinities or fresh water occur during warm seasons (Andrews 1955). Hurricane deluges in late summer and sudden ice thaws during wet springs are the usual causes of low salinities. Low-salinity mortalities depend primarily on temperature levels and duration and continuity of exposure. At winter temperatures of  $< 5^\circ\text{C}$ , oysters are quite tolerant of low-salinity conditions, either by dormancy or, if salinities are  $> 5$  ppt, by opening their shells for water exchange for respiration. Oysters are also tolerant of low-oxygen levels of down to about 1 ml/L during warm seasons. When waters become anaerobic during warm seasons, stress is rapidly increased and duration of survival is greatly decreased. Above  $15^\circ\text{C}$ , survival is limited to a few days; however, if oysters are slowly acclimated to winter dormancy, their shells remain tightly closed during freshwater exposure, and death and decomposition are slow and prolonged. The byproducts of anaerobic decomposition of meats must also affect the resiliency of the hinge ligament which causes a long delay in detecting death by open valves.

Low oxygen conditions occur in channel depths of  $\geq 8$  m every summer in the Potomac and Rappahannock rivers, but total oxygen deficiencies which cause black bottoms seldom penetrate up to the 4- to 5-m depths on oyster beds adjacent to the channels. Watermen that fish crab pots in the summer at 7- to 8-m depths near channels frequently find dead crabs in their pots that are apparently

caused by low-oxygen levels. The black bottoms on oyster beds have been observed only in the first half of May, and

not in summer when oxygen deficiencies occur regularly in deeper channels.

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**NOTE: ADDED IN PROOF** — Contribution No. 1111, Virginia Institute of Marine Science, Gloucester Point, VA 23062

## COMPARATIVE GAMETOGENIC AND SPAWNING PATTERNS OF THE OYSTER *CRASSOSTREA VIRGINICA* (GMELIN) IN CENTRAL CHESAPEAKE BAY<sup>1</sup>

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**ABSTRACT** Over a two-year period (1977-1978), samples of the eastern oyster *Crassostrea virginica* were collected nearly every month from 18 oyster beds in central Chesapeake Bay. Histological preparations of 6,309 oysters were examined and patterns of gametogenesis and spawning were determined. Early development occurred in October, with gonads remaining in that state until spring. In May, rapid gonad proliferation led to light spawning in some instances. Most animals were spawning in June, with spawning individuals present through August. In September, most individuals were in the advanced spawning or regressing stage. To compare these reproductive patterns with patterns in earlier years, we examined histological slides of 1,965 oysters collected in 1961-1963. We noted a generally similar sequence of gametogenesis, although some individuals were found in spawning condition through December in the earlier samples. We postulate that some factor(s) other than temperature (a food-related material?) may be responsible for stimulating spawning of eastern oysters in warmer waters.

### INTRODUCTION

Over the past three or four decades, especially from 1965 to 1979, natural reproductive success of the eastern oyster *Crassostrea virginica* (Gmelin) in Maryland's Chesapeake Bay (as measured by settlement of young oysters [spat] on oyster beds) was low (Krantz and Meritt 1977, personal observation). Although settlement of oysters normally varies with year and location (Beaven 1950, Engle 1955, Loosanoff 1966), the recent low level of recruitment appeared to be affecting all parts of Maryland's portion of Chesapeake Bay (Krantz and Meritt 1977).

A number of physical, chemical, or biological factors might be implicated in this decline in recruitment. In nature, the sequence of events from gametogenesis to egg release, fertilization, spat settlement, and metamorphosis may be subject to disruption, thereby leading to depressed recruitment. We initiated a study to test the hypothesis that disrupted gametogenesis and asynchronous spawning in oyster populations were contributing to the general pattern of poor settlement of spat.

We report here the results of our two-year histological study (1977-1978) of gametogenic patterns on 18 oyster beds in central Chesapeake Bay. We also examined historical material collected from the same general area of the Bay in the 1960's. The resulting data allow comparison of gametogenic patterns between the 1960's and 1977-1978. This is the first detailed, published report of such patterns in Chesapeake Bay. An evaluation of possible spawning stimuli is also included.

### MATERIALS AND METHODS

#### *Recent Histological Material*

From March to December 1977, monthly samples of about 50 oysters were collected by oyster dredge from each of 15 oyster beds in central Chesapeake Bay (Figure 1). The following year (March to September 1978), to shorten collection and processing time while expanding our sampling in the Choptank River, we collected about 25 oysters monthly from each of 14 oyster beds, 11 of which had been sampled in 1977 (Figure 1). That summer (June to September 1978), varying numbers of oysters were collected weekly from Deep Neck Bar (No. 3 on Figure 1) and Double Mills Bar (No. 6) to allow for more detailed assessment of gametogenic activity. Historically, these two beds (and the tributaries in which they are found) have differed in their levels of spat settlement, with the former region generally having a greater spatfall compared with the latter region (Krantz and Meritt 1977, Kennedy 1980).

When each station was visited throughout this survey, bottom temperature was measured using an induction salinometer. Official oyster bar names (Gird and Wheaton 1976) and brief descriptions of the 18 oyster bars sampled are found in Table 1. For purposes of later comparison, the oyster bars can be grouped within the following geographical entities: Upper Eastern Shore (Nos. 1-8), Western Shore (9-13), Lower Eastern Shore (14-18).

Preparation of oysters for histological study followed standard procedures employed by the National Marine Fisheries Service (NMFS) (Galtsoff 1964). Oysters were scrubbed, measured from the hinge to the bill (to the nearest 0.5 cm), and opened. The tissue of each oyster was then removed from the shell, rinsed in seawater, and sectioned

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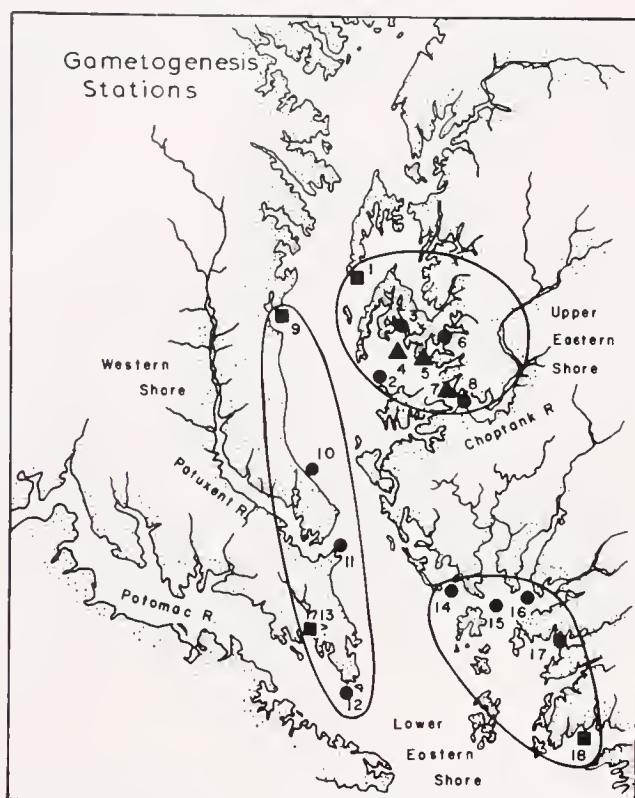


Figure 1. Location of sampling stations on oyster beds in central Chesapeake Bay (● sampled in 1977 and 1978; ■ sampled in 1977 only; ▲ sampled in 1978 only).

TABLE 1.

Descriptions of 18 oyster bars surveyed in 1977 and 1978 (as noted) for gametogenic patterns. (Numbers of oyster bars correspond to numbers on Figure 1.)

Oyster Bar	Years Sampled	Depth† (m)	Description of Spat Production
1. Hollicutt Noose*	1977	5–7	In a region with historically good spat production
2. Cook Point*	1977–78	5–8	Fair and consistent
3. Deep Neck*	1977–78	3–4	Consistently very good
4. Royston	1978	3–4	Fair to good
5. Fox Hole	1978	2–3	Fair and variable
6. Double Mills	1977–78	3–5	Poor
7. Howells Point	1978	2–6	Fair
8. Green Marsh	1977–78	3–7	Poor
9. Herring Bay*	1977	5–6	Poor
10. Flag Pond	1977–78	5–6	Poor
11. Hog Island	1977–78	2–6	Poor
12. Cornfield Harbor*	1977–78	2–3	Good to very good
13. Chicken Cock*	1977	2–3	Very good
14. Norman*	1977–78	3–6	Good
15. Sharkfin Shoal*	1977–78	2–4	Good
16. Middleground*	1977–78	2–6	Good
17. Georges*	1977–78	2–4	Fair to good
18. Marumisco*	1977	2–6	Good

\*Locations previously used by National Marine Fisheries Service and/or Maryland Department of Natural Resources for disease monitoring.

†Depths are approximate.

with a sharp razor blade. One transverse cut was made from the junction of the gills and palps, with a second transverse cut made a few millimeters below the first. The resulting segment of body tissue was fixed in Davidson's fluid, embedded, sectioned at 6  $\mu$ m, and stained in Harris' hematoxylin and counterstained in eosin.

In 1977, 25 oysters from each monthly sample from each oyster bar were selected and treated individually as above, one microscope slide being prepared per oyster. The remaining (usually 25) oysters in each sample were used in a study of sex ratios (Kennedy 1983). In 1978, each oyster was processed individually as above; however, oysters in weekly samples from Deep Neck and Double Mills bars were not measured during the summer of 1978.

The prepared microscope slides were examined to determine sex and stage of gametogenesis. In all cases, the complete gonad section of each oyster was scrutinized.

#### Historical Material

From 1961 to the present, the Maryland Department of Natural Resources and other organizations have collaborated on surveys of oyster disease, with special emphasis on the haplosporidan *Minchinia nelsoni* Haskin, Stauber, and Mackin in the 1960's (Farley 1975). Oyster tissues were collected from a number of oyster bars on or near most of those chosen for our survey (Table 1). The tissues were generally prepared as described earlier. Slides from the pathology collection (NMFS Laboratory, Oxford, MD) were examined to determine sex and stage of gametogenesis of those oysters collected in earlier years, which permitted a comparison with the conditions that existed during our 1977–1978 survey.

## RESULTS

#### Recent Histological Material

The mean size of oysters collected varied with location, but, in general, mean lengths on the different bars ranged from 9.8 to 12.9 cm in 1977 (grand mean = 11.3 cm) and from 9.6 to 12.8 cm in 1978 (grand mean = 11.1 cm). Oysters from two bars were consistently smaller than the rest and were not included in the above figures: Deep Neck oysters averaged 8.6 cm in 1977 and 8.7 cm in 1978; Sharkfin Shoal oysters averaged 8.9 cm in 1977 and 9.0 cm in 1978.

Monthly bottom temperatures measured on oyster bars were averaged within the three major locations of the study (Table 2). In general, the Lower Eastern Shore region was slightly warmer than the other two regions in spring and summer when gonadal ripening and spawning were occurring.

TABLE 2.

Average bottom water temperatures ( $^{\circ}\text{C}$ ) over oyster bars in three regions of central Chesapeake Bay, 1977–1978.

Location	1977											1978						
	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec		Mar	Apr	May	Jun	Jul	Aug	Sep
Western Shore	7.9	12.6	15.7	21.3	28.3	28.4	25.2	19.2	16.3	7.6		3.0	9.7	17.1	22.4	24.5	28.3	25.0
Upper Eastern Shore	10.4	12.4	14.8	21.2	30.2	28.9	23.8	16.6	14.8	5.6		4.8	11.4	18.8	23.8	25.2	28.5	24.6
Lower Eastern Shore	11.1	14.3	15.9	21.4	30.3	28.6	24.2	18.0	15.1	7.1		5.8	12.0	19.8	24.3	24.2	29.1	24.1

**Developmental Stages**

Loosanoff (1942) and Kennedy and Battle (1964) described the stages of gametogenic activity in *Crassostrea virginica*. Our scheme is a modification of theirs. Photomicrographs of various developmental stages of oysters can be found in papers by Loosanoff (1942, 1969), Loosanoff and Davis (1952), Kennedy and Battle (1964), and Berg (1969) and, therefore, are not presented here. Briefly, four stages of gametogenic development were defined using qualitative criteria based on gonad appearance, presence or absence of different sexual products, and evidence of sperm or egg release.

**Early Development**

Follicles are increasing in number, expanding in size, and beginning to branch, while still occupying a relatively small area of gonad. In females, a single layer of enlarging germinal cells begins to project into the lumina of the follicles. In males, germinal epithelium begins to show stratification and spermatids may be present in small numbers.

**Later Development**

Follicles have increased greatly in size and interfollicular connective tissue has decreased in quantity. As morphologic ripeness occurs in both sexes, follicles become large and distended, with the gonad occupying almost all the space between the digestive gland and the surface of the oyster body. Limited vesicular tissue remains. For females, large numbers of oocytes eventually become free in the lumina. For males, the central follicular area is occupied by large numbers of spermatozoa with their tails extending into the lumen. At full maturity, sperm masses may protrude into genital ducts preparatory to discharge.

**Spawning**

For females, follicles are discharging their ova and are contracting. Regular arrangement of oocytes is disrupted. Young oocytes may remain (with some continuing to mature) on the follicle walls. For males, sperm discharge disorganizes the lamellar arrangement of cells in the follicle lumen and may leave the follicle center empty. Initially, male follicles may not shrink greatly in comparison with female follicles. Outer bands of spermatocytes and spermatids are present and many presumably continue to mature.

**Advanced Spawning, Regressing**

Follicles become much contracted in both sexes. For females, oocytes may remain free in the lumen. In males, usually no spermatozoa remain. In both sexes, early gametogenic stages usually remain on follicle walls. Connective tissue reappears in interfollicular areas and phagocytic cells occur in increasing numbers inside and outside follicles. In our study, follicles never disappeared completely, although they usually became very small in size. Unless an old egg or pocket of male gametes remained, the sex of the oyster during the past spawning season was undeterminable.

**Reproductive Patterns**

The patterns noted for the 18 oyster bars surveyed in 1977 and 1978 are presented in Figures 2 through 5. In all, 6,309 individual oysters were examined. In the monthly samples, the early development stage generally predominated in October, with gonads remaining in that state until April or May. In May there was a rapid increase in gonad proliferation, resulting in attainment of the later development stage on many oyster bars and with initial light spawning occurring on some bars. By June, spawning was underway on all oyster bars, continuing into August. In September, although spawning was noted on some oyster grounds, most animals were in the advanced spawning or regressing stage.

For samples collected weekly in 1978 from Deep Neck and Double Mills oyster bars (Figure 2), spawning continued from middle or late June until late August (Deep Neck) or early September (Double Mills), with some individuals having reached the advanced spawning stage as early as mid-July. On 22 May, most animals from both bars were still in the early development stage. Double Mills oysters were spawning 23 days later (unfortunately, no Deep Neck oysters were collected on 14 June), demonstrating the rapidity with which oysters in central Chesapeake Bay can progress from early development to spawning (see also Table 3).

Variations from the general pattern occurred, both from bar-to-bar and from year-to-year. On Lower Eastern Shore bars (Figure 4), spawning had begun on all oyster grounds surveyed in May 1977 and 1978 (on two oyster bars a small number showed signs of spawning in March 1978, but this may have been a case of gonadal material being retained over winter). Contrarily, most oysters surveyed in May 1977 and 1978 on Upper Eastern Shore and Western Shore bars

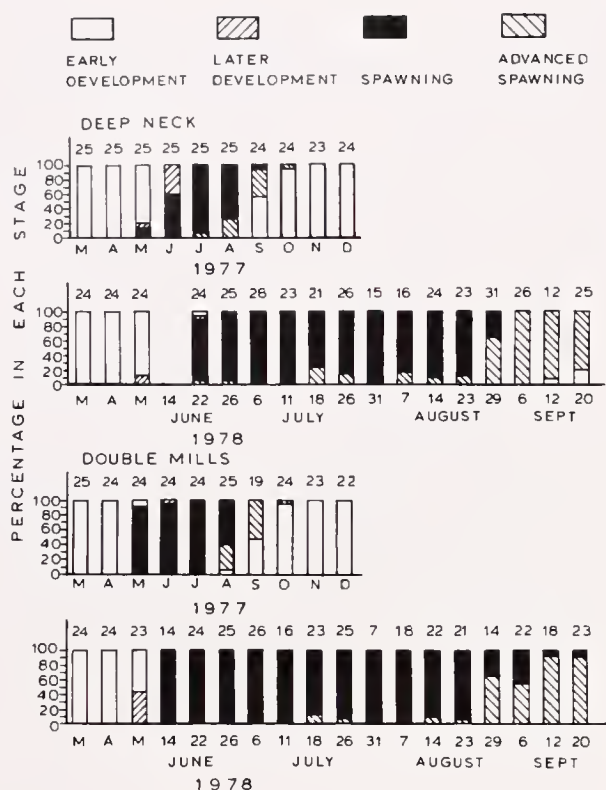


Figure 2. Percentage of individuals in each gametogenic stage over time (1977-78) for oysters from Deep Neck and Double Mills beds. For a description of the four general stages, see text. Sample size for specific month and location given above each bar.

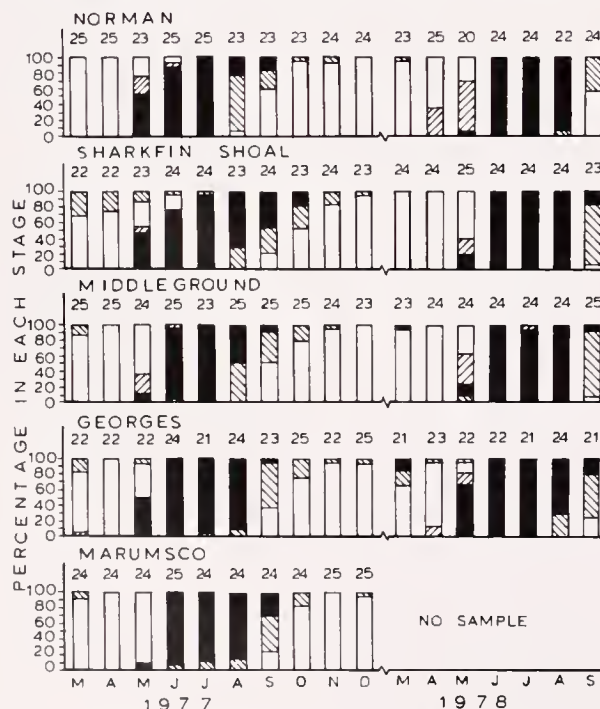


Figure 4. Percentage of individuals in each gametogenic stage over time (1977-78) for oysters from beds in the lower Eastern Shore region. (Bar shading and sample size data as in Figure 2.)

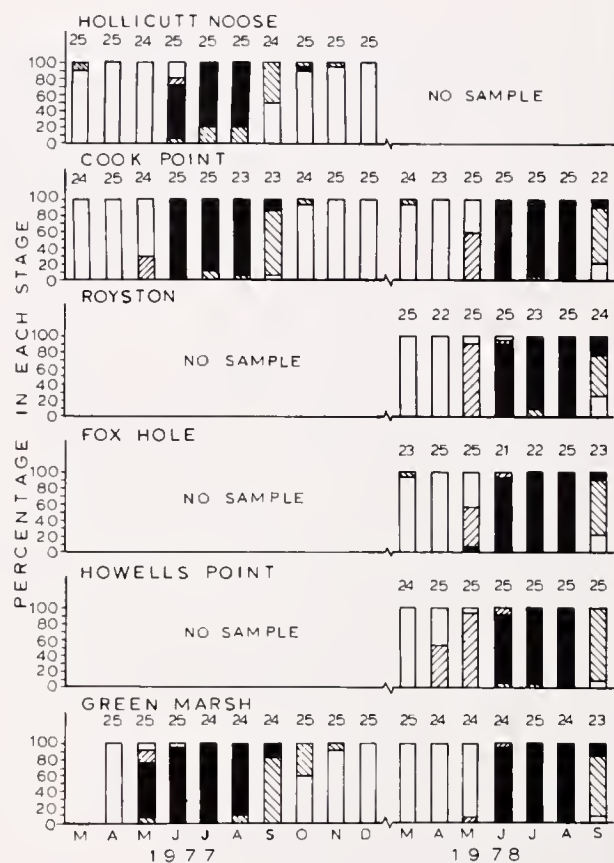


Figure 3. Percentage of individuals in each gametogenic stage over time (1977-78) for oysters from beds in the upper Eastern Shore region. (Bar shading and sample size data as in Figure 2.)

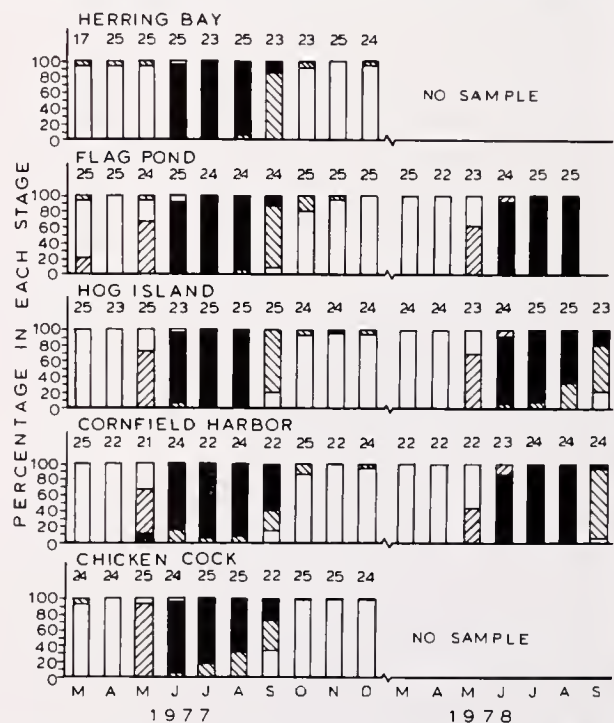


Figure 5. Percentage of individuals in each gametogenic stage over time (1977-78) for oysters from beds in the Western Shore region. (Bar shading and sample size data as in Figure 2.)

had not begun to spawn (Figures 2, 3 and 5). The exceptions were Deep Neck and Double Mills oysters in 1977 (Figure 2), Green Marsh oysters in 1977 and Fox Hole oysters in 1978 (Figure 3), and Cornfield Harbor oysters in 1977 (Figure 5).

#### Historical Material

The most continuous and complete series of prepared tissue in the disease surveys came from three areas and in collections made from 1961 to 1963. One series was from Broad Creek (no oyster bar named), the embayment in which Deep Neck oyster bar is located. A second series was from Crab Point bar which is contiguous with Norman bar. The third series came from Marumscow bar. The Broad Creek and Crab Point collections were extensive only for 1961; the Marumscow collection included many individuals collected in 1962 and 1963, thereby qualifying as a three-year survey. A total of 1,965 oysters was examined from the three regions (Figure 6).

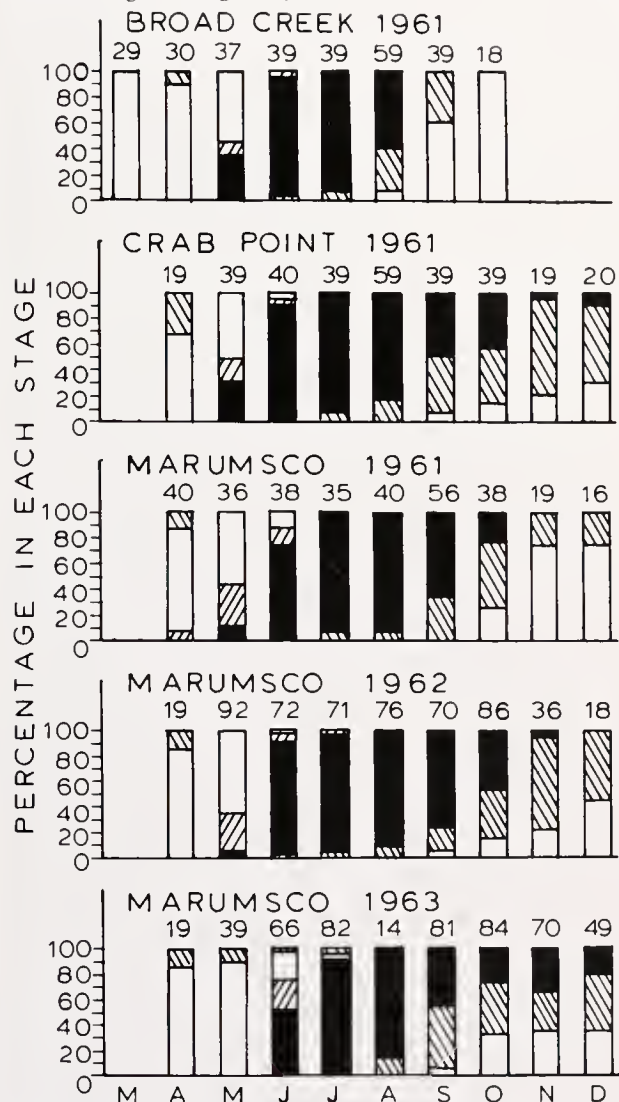


Figure 6. Percentage of individuals in each gametogenic stage over time (1961–63) for oysters from three different beds in central Chesapeake Bay. (Bar shading and sample size data as in Figure 2.)

In 1961, some spawning occurred in May on all three oyster bars (see also Marumscow bar for 1962 on Figure 6). Spawning ceased in the Broad Creek oysters by September (as it did on Deep Neck in 1978, Figure 2), but continued into December at Crab Point. This was much later than on nearby Norman bar in 1977 and 1978 (Figure 4). On Marumscow bar, spawning extended into October in 1961, November in 1962, and December in 1963; by comparison, it ceased after September in 1977 (Figure 4).

Overall, the general trend in the 1960's and in 1977–78 was for functional males and females to be found spawning in synchrony throughout the season. In a few instances, it appeared that some male oysters matured faster and initiated early spawning slightly in advance of the female oysters.

#### Gametogenesis and Temperature

Most spawning was underway in June, when mean temperatures in 1977 and 1978 ranged from about 21° to 24°C (Table 2). However, in the May spawnings, temperatures were much lower and the spawnings involved the release of limited quantities of gametes. For example, in 1977, bottom water temperatures recorded at the time of collection were 16.1°C at Double Mills, 15.0°C at Green Marsh, and averaged 15.9°C in the Lower Eastern Shore region (Table 2) where many oysters showed signs of spawning (Figure 4). In May of 1978, spawning was not as intense but occurred at a mean water temperature of 19.8°C in the Lower Eastern Shore region (Table 2).

To examine the correlation of temperature and initial spawning more closely, we compiled weekly or fortnightly temperatures measured at the time of collection of oyster samples in 1961–1962 (Table 3). (Unfortunately, we do not know if these were surface or bottom temperatures. If they were surface measurements, bottom temperatures may have been slightly lower.) In 1961, spawning began in late May on all three oyster bars when temperatures ranged from 17.6° to 19.8°C. On the other hand, while spawning on Marumscow again began by late May 1962, it occurred at 21.0°C and was preceded by a temperature of 23.8°C. Variation in recorded temperatures was also noticeable, with temperatures sometimes falling from one sampling period to the next (e.g., Marumscow bar in 1962; Table 3).

## DISCUSSION

#### Reproductive Patterns

No published reports exist concerning reproductive patterns in Chesapeake Bay, although some limited research involving aspects of gametogenesis has been reported by Truitt (1929), Butler (1949), and Bahr and Hillman (1967). In general, except for timing, the gametogenic patterns for 1977–1978 resembled those described for populations elsewhere (Loosanoff 1942, 1965, 1969; Loosanoff and Davis 1952; Kennedy and Battle 1964; Berg 1969; Price and Maurer 1971). Spawning in the Bay occurred over a

TABLE 3.  
Percentages of four gametogenic stages in relation to  
temperature ( $^{\circ}\text{C}$ ) in three locations in  
Chesapeake Bay, 1961–1962.

Location	Date	$^{\circ}\text{C}$	Gametogenic Stage			
			Early Devel.	Later Devel.	Spawning	Advanced Spawning
Broad Creek	5/10/61	19.0	95	5	0	0
	5/23/61	19.0	11	16	72	0
	6/ 6/61	22.0	0	5	90	0
Crab Point	5/ 9/61	20.0	89	11	0	0
	5/24/61	17.6	15	25	60	0
	6/ 7/61	22.5	10	10	80	0
Marumsc	5/11/61	20.1	83	17	0	0
	5/25/61	19.8	33	44	22	0
	6/ 8/61	25.9	22	33	44	0
	6/21/61	23.0	0	0	100	0
	5/16/62	18.6	82	18	0	0
	5/22/62	23.8	60	40	0	0
	5/29/62	21.0	25	50	25	0
	6/ 5/62	22.8	0	29	71	0
	6/12/62	23.7	0	19	81	0

longer period than in the cooler waters of Prince Edward Island, Canada (late June to August; Kennedy and Battle 1964), but over a shorter period than in warmer waters of Florida (March to October; Ingle 1951) or Hawaii (February to November; Sakuda 1966). The spawning period in the Bay was similar in length to that in Long Island Sound (Loosanoff 1965). In the latter region, Loosanoff (1942) noted a winter dormancy period followed by rapid maturation and found pronounced differences in maturity of oysters taken from the same place and same time, as we did.

Spawning on the Lower Eastern Shore bars extended over a longer time period in the early 1960's than in 1977–1978 (Figures 4 and 6). Truitt (1929) summarized data from 1919–1929 for Maryland waters which were probably derived from gonadal smears or inspection of shucked oysters. He noted that spawning began during the first 10 days of June and continued irregularly to late September or early October. Although there were generally two periods of peak larval abundance during the summer, Truitt (1925) noted that the more productive period of larval abundance usually occurred in August or early September; however, during the 1924 season larvae persisted into October. Beaven (1955) noted that oyster setting might occur in Maryland from late May until early October. We have no explanation for the recent apparent change in length of the spawning period; however, our two years of observation (1977–1978) may not have been long enough.

#### Spawning Stimuli

Some investigators have postulated the existence of physiological races of oysters that react to different spawning temperatures (Nelson 1928, Stauber 1950,

Loosanoff and Nomejko 1951). Truitt (1929) reported that spawning in Maryland did not occur below  $20^{\circ}\text{C}$  nor were oyster larvae present at temperatures below about  $20^{\circ}\text{C}$ . Butler (1956) reported that Maryland oysters held for a year in Florida spawned at  $25^{\circ}\text{C}$ , a level  $5^{\circ}\text{C}$  above that of the parent stock; spawning also extended over a longer period in the new environment. This demonstrates the lability of "spawning temperatures." In southern Chesapeake Bay, Loosanoff and Nomejko (1951) reported that  $20^{\circ}\text{C}$  was reached before gonad maturation occurred; spawning usually began around  $25^{\circ}\text{C}$ . As reported earlier, however, we found that some oysters initiated spawning early when water temperatures were below  $20^{\circ}\text{C}$ .

Care is needed in drawing conclusions from all of these facts because temperatures may vary rapidly in these shallow, hydrographically dynamic, estuarine environments. Price and Maurer (1971) used a day-degree approach in an effort to integrate past thermal history and avoid reliance on single temperature measurements collected at irregular or widely spaced intervals.

Factors other than temperature may be involved in triggering spawning. Although temperature is manipulated to spawn conditioned oysters in hatcheries (Loosanoff and Davis 1963), oysters from areas south of New Jersey are more difficult to condition and spawn (Loosanoff and Nomejko 1951, Hidu et al. 1969, Loosanoff 1969, Andrews 1979). Appropriate food and appropriate temperature conditions are necessary (Hidu et al. 1969, Dupuy et al. 1977).

Butler reported (in Nelson 1955, 1957) that water temperatures at Pensacola, FL, often reached  $25^{\circ}\text{C}$  for a number of weeks prior to the spawning of local oysters; apparently a spring phytoplankton bloom was necessary to stimulate spawning. Nelson (1955) also noted that Long Island Sound oysters had not spawned in July 1954 even though temperatures were sufficiently high (see also Roughley [1933] concerning Australian oysters). Nelson (1955, 1957) speculated that some sort of material (a vitamin, or pectin, or other carbohydrate) might be released by phytoplankton, thus stimulating oyster spawning. Among other molluscs, five chiton species on the western coast of North America spawn in the spring when phytoplankton populations are increasing (Himmelman 1980). Thorson (1936) found that two species of Greenland bivalves spawned during a phytoplankton bloom before temperatures increased.

Breese and Robinson (1981) stimulated a number of bivalves (including *Crassostrea gigas* and *C. rivularis*) to spawn by holding them in concentrations of phytoplankton species; traditional methods of eliciting spawning (temperature change and chemical stimulation) had previously been unsuccessful. The nature of the stimulant responsible for these responses and its presence and activity in the wild remain to be determined; however, Miyazaki (1938) found a substance in *Ulva* sp. that stimulated spawning in *Crassostrea gigas*, at a concentration of 1 ml of a 1 ppt solution.

With these findings in mind, and with regard to *Crassostrea virginica* which extends from cold temperate waters in Canada to the warm subtropic Gulf of Mexico, we hypothesize that different triggering mechanisms operate from one part of its range to another. Specifically, in colder waters with limited periods of high temperature, it may be advantageous to respond to a temperature increase rather than to a food stimulus or a temperature-food combination. The latter stimuli may be delayed in occurring, yet the time available for larval development is short and spawning delays might not leave sufficient time for complete growth to metamorphosis. Contrarily, in warmer environments this time period is much longer and it may, therefore, be advantageous to respond to the presence of suitable food material to enhance larval survival. Presumably, any delay in the occurrence of a suitable phytoplankton bloom in warmer waters may be less disastrous than it might be in colder waters where a few weeks rather than a few months serve as the "window" for larval development through metamorphosis.

### CONCLUSIONS

This survey of reproduction on central Chesapeake Bay oyster grounds in 1977–1978 has shown that gametogenesis apparently was not disrupted and that synchronous

spawning occurred. Recent declines in spatfall success are probably not attributable to these factors as we proposed at the start of our study. Except for the length of spawning period, which was less than it had been in the 1960's in the more southerly part of our study region, the recent reproductive patterns were similar to those of the 1960's. From our data and from reports in the literature, we hypothesize that the presence of a food-related chemical or some temperature-food stimulus may initiate spawning in these warm temperate waters, rather than temperature alone.

### ACKNOWLEDGMENTS

We thank Dr. G. Krantz for helping us in the initial stages of this study, which emerged from his own thinking about the problem of low recruitment. D. Smawley, J. Connor, M. O'Berry, and M. Reusing assisted in the field. Dr. A. Rosenfield and Ms. S. Otto provided access to the historical material. Dr. R. Newell reviewed an earlier draft of this report. D. Kennedy drew the figures. Dr. J. Andrews of the Virginia Institute of Marine Science provided insight into the subject during various discussions. Financial support was provided by the University of Maryland Sea Grant Program under Grant Nos. 04-8-M01-73 and NA79AA-D-00058.

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## CONTAINERIZED-RELAYING OF POLLUTED OYSTERS (*CRASSOSTREA VIRGINICA* [GMELIN]) IN MISSISSIPPI SOUND USING SUSPENSION, RACK, AND ONBOTTOM-LONGLINE TECHNIQUES

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**ABSTRACT** Two polyethylene containers designed for transporting chickens were adapted for relaying (cleansing) commercial quantities of polluted oysters (*Crassostrea virginica*). The 86- X 56- X 20-cm Piper coop (Piper Industries, Jackson, MS), which has a hinged lid and holds one sack (0.03 m<sup>3</sup>) of oysters, was suspended in three-coop stacks in approved shellfish growing waters. During five experiments, oysters purged fecal coliform (indicator) bacteria from initial levels of  $\leq 1,400$  MPN/100 g to or below the recommended level of 50/100 g within the required 15-day relaying period. During three onbottom, longline-relaying experiments with Piper coops, oysters purged fecal coliforms from 2,800/100 g to  $\leq 50/100$  g within 15 days, provided that the bottom was firm enough to prevent settlement and burial of the coops and oysters. The 86- X 56- X 10-cm Phillips coop (bottom) (Phillips Petroleum Co., Henderson, KY), which has no lid but holds one sack of oysters, was used in three offbottom experiments with a patented, metal rack (E. R. Gollott, Biloxi, MS) which holds 48 coops in a sliding-tray arrangement (6 coops X 2 rows X 4 levels). Oysters from eight coops in various positions and levels purged fecal coliforms from 23,000/100 g to  $\leq 50/100$  g within 10 days. Cleansing success depended primarily on sustained, approved water quality; however, the experiments demonstrated that the container type, the relaying method, and the initial condition of the oysters were also important factors. Rack-relaying with the Phillips coop resulted in mean mortalities of only 1.6% compared with  $> 70\%$  using conventional, onbottom (unprotected) relaying techniques. Containerized-relaying has immediate applicability to commercial relaying in Mississippi. Polyethylene coops reduce predation, ensure complete harvests, and serve as acceptable relaying, transport, wash-down, and storage containers throughout the relaying process.

### INTRODUCTION

Oysters concentrate biological pollutants from contaminated estuarine waters in the gut and digestive gland via filterfeeding and thereby become a potential health hazard (Old and Gill 1940, Mason and McLean 1962, Metcalf and Stiles 1956). When placed in uncontaminated waters for a few days, however, oysters purge themselves of pollutants, resulting in a safe, edible product (Furfari 1966, Cook and Childers 1968, Neilson et al. 1976).

The acceptable methods of cleansing are onbottom relaying and onshore depuration (Houser 1965). Onbottom relaying (transplanting oysters from "closed" reefs to approved shellfish growing waters) results in high losses because of: (1) physiological stress; (2) shell damage inflicted by relaying; (3) smothering and clogging by sediments; (4) predators; and (5) incomplete second harvests. In onshore depuration, contaminated oysters cleanse themselves within 48 to 72 hours while exposed to ultraviolet- or ozone-treated seawater in specifically designed facilities. Those facilities are generally expensive to construct, maintain, and operate. After each cleansing method, fecal coliform analyses are required to confirm that cleansing has been accomplished (Ratcliffe and Wilt 1974).

A third alternative, "containerized-relaying," involves the use of a "relaying device" (e.g., raft, rack, etc.) to hold or suspend containers or baskets of oysters on or off the bottom in approved waters until they are cleansed. Harvesting is accomplished by simply lifting the containers of "clean" oysters from the water. This method alleviates some of the logistic problems of onbottom relaying while reducing

bottom suitability as a limiting factor. Containerized-relaying may also be more economically feasible than onshore depuration.

Previous depuration research (Kelley et al. 1960, Furfari 1966, Presnell et al. 1968, Neilson et al. 1976) provide optimum hydrological conditions. A water temperature range of 10 to 29°C is optimum for self-cleansing. Temperatures below 10°C inhibited successful cleansing. A salinity range of 14 to 22 ppt is ideal. Furfari (1966) recommended that depuration waters not exceed 20 Jackson Turbidity Units (JTU). Presnell et al. (1968) and Neilson et al. (1976) reported, however, little or no difference in depuration with turbidities as high as 69.4 and 77 JTU, respectively.

The objectives of this study were: (1) to determine the effect of containerized-relaying on oyster survival and purification, [a] when the oysters were moved during different seasons (under different physiological conditions), [b] under different salinity and temperature regimes, and [c] when different quantities of oysters were placed in various types and numbers of containers; and (2) to investigate various designs of rafts, racks, etc., for commercial relaying.

### MATERIALS AND METHODS

Experiments using the American oyster *Crassostrea virginica* (Gmelin) were conducted during pre- and post-spawning periods and during the local oyster harvesting season (September through April). The selection of specific experimental dates depended on preliminary hydrological, physiological, and environmental evaluations (i.e., correct salinities, oyster condition, weather, etc.). The experiments

began September 1978 and continued through August 1980.

Oysters used in this study were initially harvested from the following "prohibited" reefs: Pascagoula, Graveline Bayou, and Biloxi Bay (Figure 1). Oysters were relaid the same day as harvest, weather permitting, or kept overnight under wet burlap. Oysters were relaid without acclimation to determine the effects of salinity changes on cleansing and survival.

Bacteriological and physiological measurements were made on oyster samples immediately after the initial harvest and on samples of relaid oysters collected at specific intervals (days). Bacteriological and hydrological data were determined from water samples (surface and/or bottom) collected at the same time oysters were harvested. Oyster mortality data were taken at the completion of the relaying period.

Fecal (FC) coliform bacteria in oyster and water samples were enumerated using the five-tube, most probable number (MPN) technique outlined in APHA (1970). Two-hundred-gram samples of oyster meats were used, except when subsampling from containers; in those cases, three 100-g samples were used (top, middle, and bottom). Bottom water samples were collected with a device similar to the J-Z water sampler

(Zobell 1946) with adaptations described by Cook (1969). Surface water samples were collected with a sterile bottle. Oysters were considered cleansed when their fecal coliform level was reduced to or below 50/100 g. This level is the operational standard for the depuration of soft-shell clams (*Mya arenaria* Linné) in New England, which was proposed for acceptance at the 7th National Shellfish Sanitation Workshop in 1971 (Ratcliffe and Wilt 1974).

Immediately after the initial harvest and at the end of the relaying period, composite samples of 25 oysters were analyzed for:

1. Condition Index (Hopkins 1949), an indication of oyster quality, calculated as:

$$C = \frac{\text{mean dry weight (g) of meat}}{\text{volume of shell cavity (ml)}} \times 100.$$

2. The Percent Spawnability, which was determined by microscopic examination of a gonadal smear (Ogle 1979) and calculated as:

$$PS = \frac{\# \text{ oysters with gametes}}{\text{total \# oysters examined}} \times 100.$$

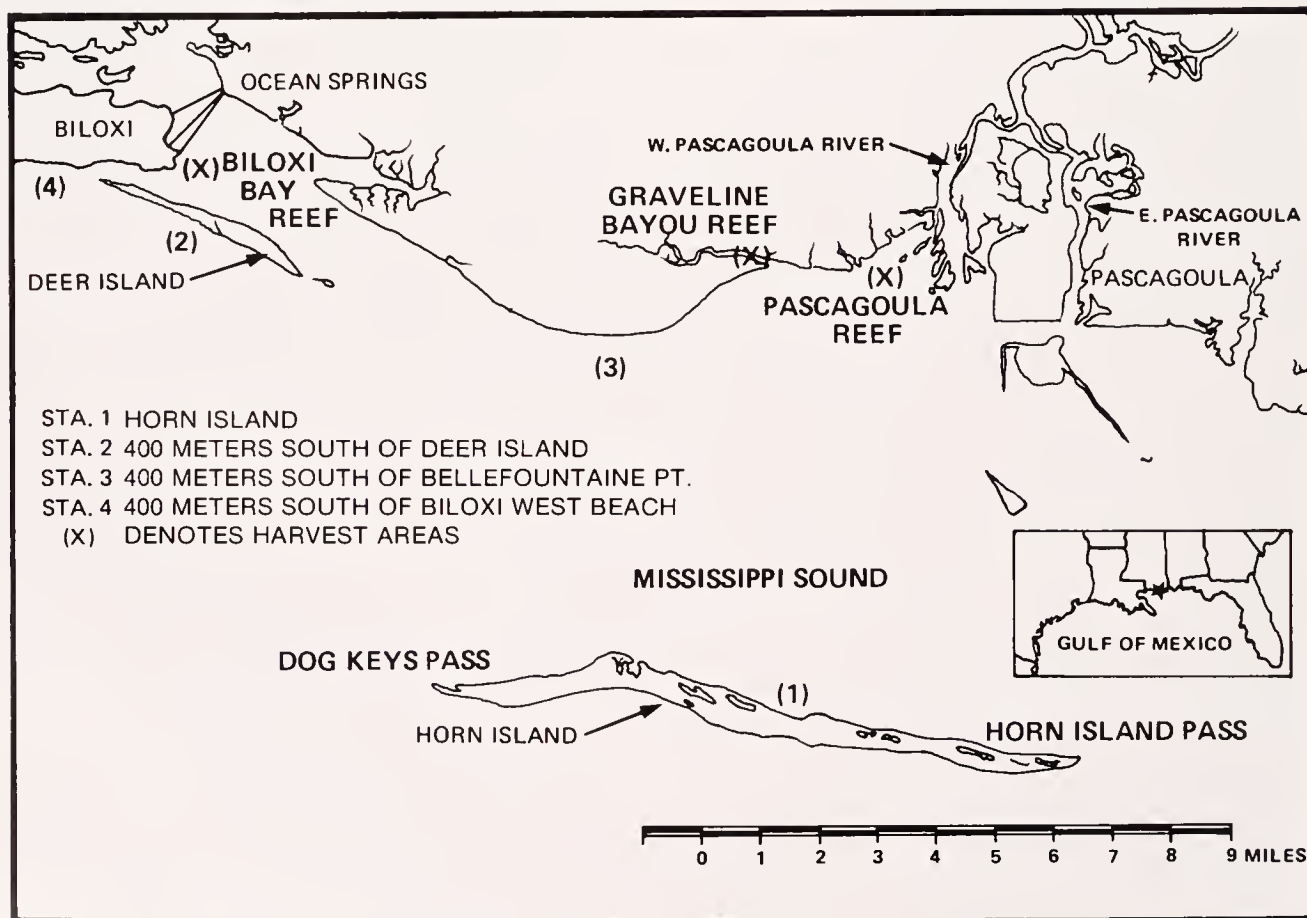


Figure 1. Map of the study area and station locations.

3. *Perkinsus* (Syn. *Dermocystidium*) *marinus* infection, using the fluid thioglycollate culture method (Ray 1952, 1966), determined by Quick (1966) as: (a) the incidence of infection, a percent expression of the proportion of the oysters tested that were positive; and (b) the weighted incidence, a numerical value expressing the average of all of the intensity code numbers (0 = negative, 1 = very light, 2 = light, 3 = light medium, 4 = medium, 5 = medium heavy, 6 = heavy).

One sack (approximately 0.03 m<sup>3</sup>) of onbottom and experimental oysters was used to estimate the percent mortality, calculated as:

$$PM = \frac{\# \text{ dead oysters}}{\text{total \# oysters counted (1 sack)}} \times 100.$$

Salinity, temperature, and turbidity measurements were made on all water samples. Bottom water samples were collected with a Kemmerer water sampler. Salinity was determined using a refractometer (Model AM125, AO Instrument Co.). Temperature was recorded with a hand-held mercury thermometer. Turbidity was estimated with a turbidimeter (Model 2100 A, Hack Chemical Co.).

The relaying of oysters was conducted at four locations in Mississippi Sound (Figure 1). Stations were chosen so as to obtain data from: (1) "high," "moderate," and "low" salinity areas; (2) different locations which might be feasible for future relaying (because of their proximity to contaminated reefs); and (3) various container usage (i.e., relaying device). Each station received one sack (approximately 0.03 m<sup>3</sup>) of onbottom relaid oysters to serve as a control. The control oysters were relaid (thrown overboard) near the experimentally relaid oysters and were retrieved with oyster tongs or by skin diving.

Station 1, north of Horn Island, MS, was chosen as a "high" salinity area. Station 2, south of Deer Island, was chosen as the "moderate" salinity area. Station 3, south of Bellefountaine Point, was chosen as the "low" salinity area and was a previous relaying area for the Mississippi Bureau of Marine Resources (MBMR) (Cook 1969). Station 4, south of west Biloxi beach was a "moderate" salinity area and was recently planted with cultch material (clam shells) by the MBMR.

The success of containerized-relaying of contaminated oysters depends upon the type of container used. Various containers were tested to devise a suitable system for the commercial cleansing, storing, and shipping of oysters and to maximize handling efficiency. At Station 1, bagged, single- and multilayer arrangements of oysters were studied with the use of: (1) 2-cm (mesh size) Vexar® bags (60 × 85 cm); (2) 56- × 56- × 5-cm, stackable, plastic Nestier® shellfish trays; and (3) 86- × 56- × 20-cm, stackable, plastic "chicken coops" (Piper Industries, Jackson, MS) (Figure 2). All containers, except the Vexar® bags, were stacked in threes and were filled with oysters. The solid-bottom "coops," which

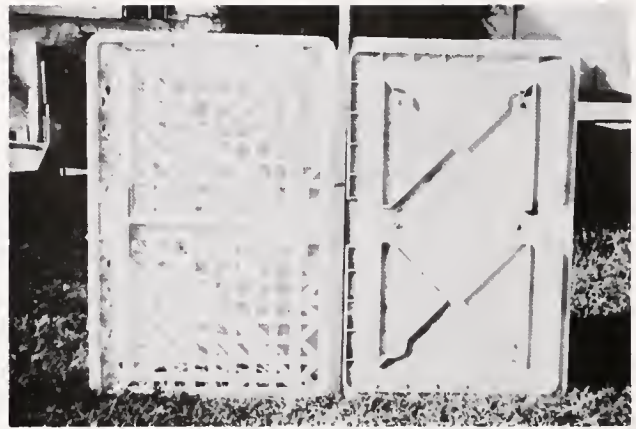


Figure 2. The "Piper" coop.

held commercial quantities of oysters (1.0 to 1.5 sacks), were used to examine the interruption of vertical flow between the top, middle, and bottom positions. Interruption of vertical flow may reduce ingestion of purged indicator bacteria and/or pseudofeces by oysters in the lower containers, but could reduce water availability for adequate cleansing. Generally, the bottom layer of oysters from the middle of each container was sampled; however, sometimes the top and/or middle layers were tested to determine if a "cleansing gradient" existed in the containers. All of the containers were suspended from a tide gauge platform with 1.25-cm (diameter) nylon rope into the water at a depth of 1 m below mean sea level.

The suitability of solid-bottom "coops" was examined at Stations 2, 3, and 4 for onbottom-relaying. That system consisted of a 1.25-cm (diameter) nylon rope (longline) attached by chain to a piling. The containers of oysters were connected to loops along the rope; they were individually thrown overboard and settled right-side-up on the bottom. Once the chain was dropped to the bottom of the piling, there was no indication of the containers; they were subsequently removed by retrieving the chain by boat hook and pulling up the longline. Periodic sampling was conducted by skin diving to remove oysters from within the "coops" for bacteriological analyses. Along with other previously mentioned parameters, sedimentation rates and settling of the containers were noted.

An "oyster rack" designed and patented by Mr. E. R. Gollott (Cap'n Gollott Seafood Co., Biloxi, MS) was deployed at Stations 2 and 3. The 3.6- × 1.8- × 1.2-m rack, constructed primarily of welded angle iron, was designed to accommodate forty-eight, 86- × 56- × 10-cm plastic trays (chicken coop bottoms, Phillips Petroleum Co., Henderson, KY) (Figure 3) in a sliding-tray format (Figure 4). The trays, with foraminated bottoms, each held one sack of oysters and were placed in a 6-tray × 2-row × 4-level arrangement with a 5-cm space between adjacent levels. The rack was supported off the bottom by two 360- × 5- × 5-cm timbers



Figure 3. The "Phillips" tray.

beneath a single 260- × 80- × 1.9-cm plywood sheet. The trays were placed into the rack, transported via barge to a station, and lowered onto a firm mud bottom in approximately 3 m of water. Oyster samples were taken from the submerged rack by skin diving. Eight trays were sampled to determine if representative areas of the rack had cleansed properly (Figure 4).

### RESULTS

The results of this study appeared to be a function of specific factors that helped or hindered successful cleansing. The physiological data were good indicators of relaying stress and seasonality (i.e., pre-, post-, and spawning seasons) on the oysters' conditioning. At no time during any of the experiments did the weighted incidence of *Perkinsus marinus* reach 4.0 units, the value of potential lethal dose (Quick 1966).

#### Container Studies (Suspension relaying)

The container studies at Station 1 produced varied results; however, they confirmed that commercial quantities of oysters in multilayers will adequately eliminate indicator bacteria.

Some FC values of relaid oysters were below recommended levels (50MPN/100 g) during the first 15-day relaying period. All oyster samples from the containers had FC values of  $\leq 45/100$  g (Table 1). The second and third experiments were marred by failures of the suspension rope, with unsuccessful cleansing in the top and bottom coops. The results of those experiments indicated that sediment-intake may deter proper cleansing. This was apparent in the successful results ( $\leq 50/100$  g) from the middle coop, which rested on top of a buried coop during both experiments. Successful cleansing occurred in most of the containers during the fourth experiment, while coop 1 had a slightly higher FC value of 70/100 g in the middle and bottom layers (Table 2).

Generally, water of approved quality (FC  $\leq 14$ MPN/100 ml) was present near Horn Island. This was apparent in all water samples taken during the first two experiments. Certain samples taken during the following three relaying periods produced high FC MPN values (Table 2). The highest FC value of the relaying waters was 2400/100 ml, followed by 10/100 ml five days later.

The hydrological factors at Station 1 were generally stable through all five experiments. The mean salinity difference between the harvest and relaying areas was 10 ppt, with a range of 5 to 15 ppt. The greatest overall difference was 19 ppt. The coldest temperatures ( $\leq 9^{\circ}\text{C}$ ) were experienced during January and February (Table 2). The mean turbidity at Station 1 was 6.5 JTU.

The condition of the oysters also varied during the first five experiments. A drop of 2.9 units in the Condition Index (CI) was followed by a decrease of 46% in the Percent Spawnability (PS) (October-November) (Table 1). The CI and PS were relatively constant through the second and third experiments with a 1.0 increase in the CI during the fourth (Table 2) (January-February).

The unsuccessful experiment # 5 (drastic increase in fecal coliforms) resulted from poor oyster conditioning (initially, some oyster valves could be pulled apart by hand). A low CI of 4.0 units throughout the trial was a good indication of this. That stress could account for the 30% and 0.35 unit increase in the incidence of *Perkinsus marinus*. Also, the use of smaller, Pascagoula Bay oysters resulted in higher packing densities, increasing the chances of adverse crowding.

Although Station 2 exhibited the highest salinities, no correlation existed between the presence of *Perkinsus marinus* and successful cleaning during the five relaying periods. Oysters in the first two experiments exhibited high percent incidences of infection (50% and 72%, respectively). The widest single experimental range between the initial and final values was 30%. The weighted incidence of *Perkinsus* was  $\leq 1.0$  during all five experiments.

Multilayering in the coops did not affect oyster mortality. Except for equipment failure, oyster mortalities in the coops

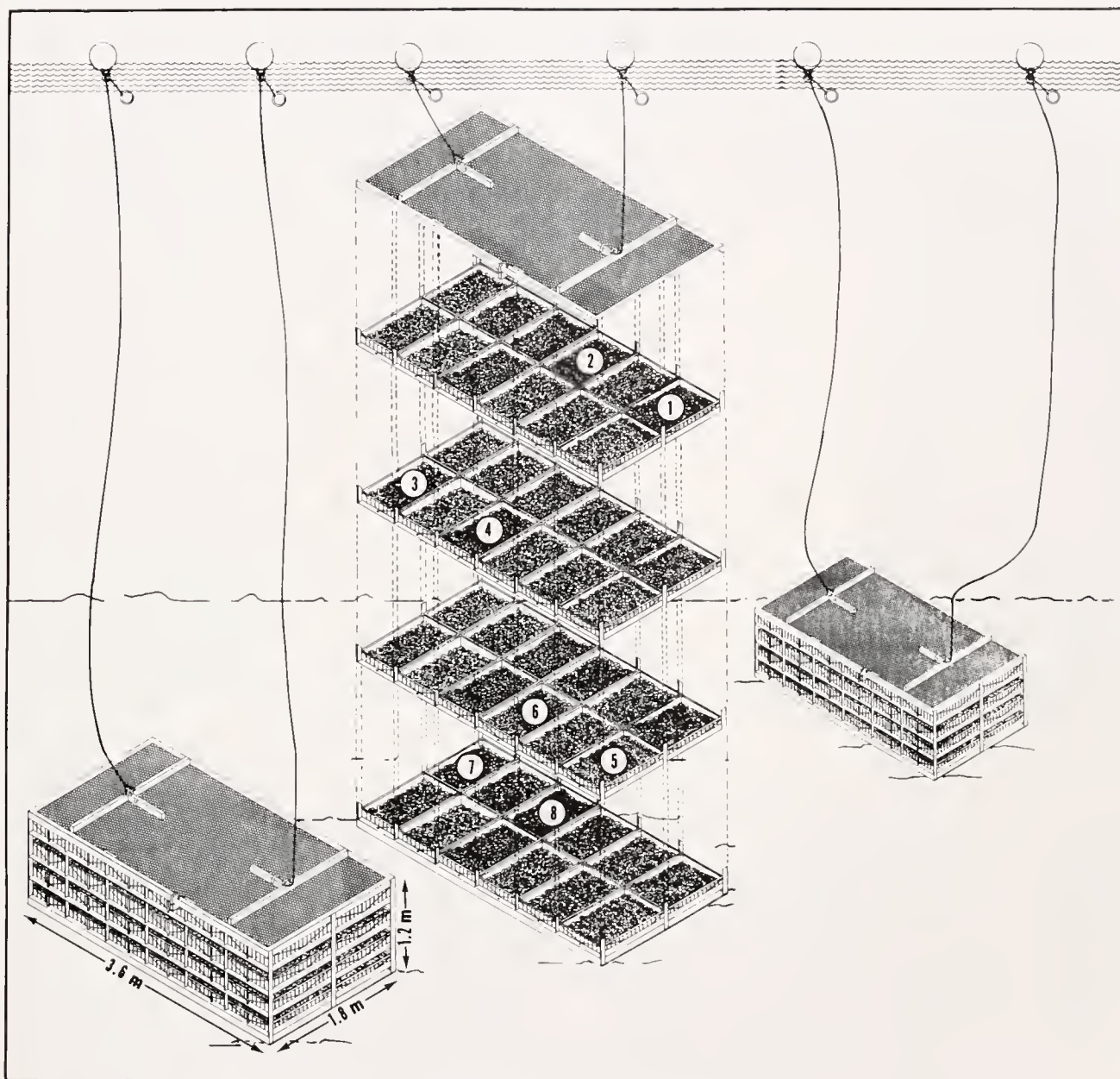


Figure 4. The "oyster rack" with location of sampled trays.

were generally low, averaging 8.5% through all five experiments. The fifth experiment had the highest mortalities (12.4 to 16.5%). The high mean mortalities among onbottom control oysters (35%), resulted from poor bottom suitability (shifting sand) of the Horn Island area.

#### *Onbottom relaying (Longline)*

Acceptable reductions in the FC MPN values occurred in the longline-relaying studies. Fecal coliforms were reduced approximately 2800/100 g from the initial value after 14 days (Table 3). The second experiment was unsuccessful after 7 and 13 days. Fluctuations occurred between the top

and bottom samples of the coops during that relaying period. During the third experiment; initial FC values dropped from 350 to 45/100 g during the first seven days.

Water quality was generally acceptable; however, poor water quality conditions of 17 and 20 MPN/100 ml (FC) occurred on two occasions at Station 3.

Hydrological factors were favorable during the three experiments. The oysters experienced a gradual increase of water salinity to 25 ppt over 13 days during the second experiment. Water temperatures were high, averaging approximately 25°C, for all three relaying periods. Turbidity values of 50 to 55 JTU were common.

TABLE 1.  
Container Study, Horn Island (Station 1)

Date/Day	Sample <sup>1</sup>	FC Coliform (/100 g or ml)	Sal. (ppt)/ Temp. (C)	Turbidity (JTU)	Cond. Index	% Spawn	<i>Perkinsus</i> % Inc./ Wt. Inc.	% Mort.
10-20-78/0	Oysters (H)*	640			6.9	48	36/0.5	
	Water (H)*	110	24/22	11				
10-22/0	Water (R)*	2	31/21	2				
11-1/10	Water (R)*	2	31/23	2				
11-6/15	Water (R)*	2	31/22	3				
	Oysters (R)				4.0	4	50/0.5	
	Vexar Bag	20						14.3
	Tray 1	20						6.1
	Tray 2	20						2.2
	Tray 3	40						2.2
	Coop 1(T)	45						6.5
	Coop 1(B)	20						6.5
	Coop 2(B)	20						13.5
	Coop 3(B)	20						8.0
	Oysters (C)				4.0	4	50/0.5	38.3

<sup>1</sup> Sample Code:

H = Harvest Area

R = Relaying Area

C = Onbottom Control Oysters

\* = Average of Two Samples

Tray 1 = Top Nestier Tray

Tray 2 = Middle Nestier Tray

Tray 3 = Bottom Nestier Tray

Coop 1 = Top Piper Coop

Coop 2 = Middle Piper Coop

Coop 3 = Bottom Piper Coop

TABLE 2.  
Container Study, Horn Island (Station 1)

Date/Day	Sample <sup>1</sup>	FC Coliform (/100 g or ml)	Sal. (ppt)/ Temp. (C)	Turbidity (JTU)	Cond. Index	% Spawn	<i>Perkinsus</i> % Inc./ Wt. Inc.	% Mort.
1-26-79/0	Oysters (H)*	900			10.7	0	10/0.2	
	Water (H)*	790	15/8	5				
1-29/0	Water (R)*	89	20/9	7				
2-3/5	Water (R)*	2	26/8	7				
2-8/10	Water (R)*	70	18/7	10				
2-12/14	Water (R)*	17	20/9	8				
	Oysters (R)				11.7	0	10/0.2	1.8
	Vexar Bag	Lost						
	Coop 1(T)	50						1.8
	Coop 1(M)	70						1.8
	Coop 1(B)	70						0.5
	Coop 2(T)	50						0.5
	Coop 2(M)	20						0.5
	Coop 2(B)	20						4.9
	Coop 3(T)	40						4.9
	Coop 3(M)	20						4.9
	Oysters (C)				11.7	0	10/0.2	17.3

<sup>1</sup> Sample Code:

H = Harvest Area

B = Bottom Layer of Oysters

R = Relaying Area

\* = Average of Two Samples

C = Onbottom Control Oysters

T = Top Layer of Oysters

M = Middle Layer of Oysters

Coop 1 = Top Piper Coop

Coop 2 = Middle Piper Coop

Coop 3 = Bottom Piper Coop

TABLE 3.  
Onbottom-Relaying (Longline) Study, Bellefontaine Pt. (Station 3)

Date/Day	Sample <sup>1</sup>	FC Coliform (/100 g or ml)	Sal. (ppt)/ Temp. (C)	Turbidity (JTU)	Cond. Index	% Spawn	<i>Perkinsus</i> % Inc./ Wt. Inc.	% Mort.
4-29-80/0	Oysters (H)*	2800			12.3	100	18/0.18	
	Water (H)*	640	19/20	25				
4-30/0	Water (R)*	2	20/21	60				
5-7/7	Water (R)*	2	21/22	17				
	Oysters (R)							
	Coop 1(T)	78						
	Coop 1(B)	78						
	Oysters (C)	20						
5-10/10	Water (R)*	2	15/22	10				
	Oysters (R)							
	Coop 2(T)	20						
	Coop 2(B)	61						
	Oysters (C)	82						
5-14/14	Water (R)*	2	20/24	13				
	Oysters (R)				10.3	92	12/0.18	
	Coop 3(T)	20						12.8
	Coop 3(B)	20						12.8
	Coop 1							15.9
	Coop 2							9.0
	Oysters (C)	20			10.3	92	12/0.18	6.7

<sup>1</sup>Sample Code:

H = Harvest Area

R = Relaying Area

\* = Average of Two Samples

C = Onbottom Control Oysters

T = Top Layer of Oysters

B = Bottom Layer of Oysters

Coop = Piper Coop

Oysters that were sampled during all three experiments simultaneously dropped in Condition Index (CI) and Percent Spawningability (PS). In May, the CI and PS decreased by 2.0 units and 8%, respectively (Table 3). Similar CI values were recorded during the third relaying period (June-July), but a larger PS decrease of 40% occurred. The greatest CI and PS decreases were 4.1 units and 68%, respectively (August).

The incidence of *Perkinsus marinus* was not a limiting factor. The highest percent and weighted incidence were 60% and 1.5 units, respectively.

Mortalities experienced during the longline-relaying experiments were the highest of the entire study. Mortalities averaged 12.5% of the experimental oysters (Table 3). The high mortalities resulted from burial of oysters in the sediments. The third experiment was performed on a shell bed at Station 4 to help eliminate burial; however, the mean experimental mortality was 17.4%. A low Condition Index of 4.9 units was indicative of poor oyster health. The presence of a shell bed helped reduce the control mortality to 4.1%, the lowest during the entire study.

#### Rack-Relaying Experiments

The "oyster rack" had the most consistent cleansing of the three methods tested (suspension-, rack-, and longline-

relaying). All of the experiments resulted in successful cleansing, but over different intervals. Median FC MPN values of relaid oysters increased 80/100 g from day 3 to day 7 during the first experiment (of the eight trays sampled [Figure 4], five exhibited increased FC MPN values). During the second experiment, the 10-day median FC value also increased 31/100 g (Table 4). The third experiment was the most successful. Fecal coliforms decreased from 23,000 to 20/100 g after 10 days (Table 5).

Approved water quality existed during all three experiments. The only exception was a FC value of 32/100 ml on day 3 of the first experiment. Fluctuating bacteriological water qualities affect self-cleansing. During heavy rainfall and "northers" (strong northerly winds), particularly in Mississippi Sound, water quality is reduced. Land runoff increases fecal coliform counts, which result in slower oyster cleansing or recontamination (Cook 1969). This reinforced the present classification of Mississippi Sound as conditionally approved (i.e., FC values greater than 14 MPN/100 ml at times).

The fluctuating hydrological factors were intrinsic to the particular relaying area. The widest salinity range during a single experiment (#1) was 27 ppt and occurred at Station 3. Differences of 10 to 15 ppt occurred between the harvest

TABLE 4.  
Rack-Relaying Study, Deer Island (Station 2)

Date/Day	Sample <sup>1</sup>	FC Coliform (/100 g or ml)	Sal. (ppt)/ Temp. (C)	Turbidity (JTU)	Cond. Index	% Spawn	<i>Perkinsus</i> % Inc./ Wt. Inc.	% Mort.
11-28-79/0	Oysters (H)*	1400			9.5	0	28/0.4	
	Water (H)*	1200	0/17	17				
	Water (R)*	33	15/17	7				
12-5/7	Water (R)*	2	22/10	5				
	Oysters (R)				9.7	0		
	Tray 1	78						
	Tray 2	45						
	Tray 3	45						
	Tray 4	20						
	Tray 5	110						
	Tray 6	20						
	Tray 7	20						
	Tray 8	45						
	Median	45						
	Oysters (C)*	20			9.7	0		
12-8/10	Water (R)*	1	26/13	17				
	Oysters (R)				10.8	0		
	Tray 1	110						
	Tray 2	78						
	Tray 3	18						
	Tray 4	78						
	Tray 5	78						
	Tray 6	45						
	Tray 7	20						
	Tray 8	20						
	Median	61						
	Oysters (C)	20			10.8	0		
12-14/16	Oysters (R)				12.0	0	44/0.4	1.6
	Oysters (C)				12.0	—	44/0.4	19.0

<sup>1</sup> Sample Code:

H = Harvest Area

\* = Average of Two Samples

R = Relaying Area

Tray = Phillips Coop

C = Onbottom Control Oysters

and relaying areas during all experiments (Tables 4 and 5). Water temperatures were low and reached 10°C during experiments 2 and 3. Turbidities ranged from 3 to 35 JTU.

The oysters were in excellent condition during all three experiments. An increase in the Condition Index (CI) of 2.7 units occurred during experiment # 1 (November); little or no change was observed in the Percent Spawnability (PS). The CI during experiment # 2 (December) also increased, by 2.5 units (Table 4). Spawning apparently occurred during the third experiment (February), with simultaneous decreases in the CI (2.0 units) and PS (84%) after 10 days (Table 5).

The lowest mortalities of container-relaid oysters occurred during rack-relaying studies (mean mortality, 1.3%). Those mortalities were 30% lower than the control mortalities (19 to 30%).

#### DISCUSSION

If approved water quality in the relaying area is sustain-

able, the factors that determine successful cleansing are: (1) oyster physiology, (2) the design of the container, and (3) its method of use (i.e., raft, rack, longline, etc.). All types of containers used in this study were acceptable in that they permitted the oysters to reduce fecal coliform bacteria to or below recommended levels. The multilayering of oysters in the "Piper" and "Phillips" coops is permitted by state and federal health agencies in Mississippi. The time required for oysters to cleanse varied because of recontamination, container burial, and poor oyster condition. Oyster mortality was also dependent on the latter two factors.

#### Previous Containerized-Relaying Studies

Becker (1977) conducted an offbottom, containerized cleansing study in eastern Mississippi Sound using stainless steel baskets (35.5 cm/side). He found that indicator bacteria were effectively eliminated from single-layered oysters in 24 h when ambient water quality met or exceeded the

TABLE 5.  
Rack-Relaying Study, Deer Island (Station 2)

Date/Day	Sample <sup>1</sup>	FC Coliform (/100 g or ml)	Sal. (ppt)/ Temp. (C)	Turbidity (JTU)	Cond. Index	% Spawn	<i>Perkinsus</i> % Inc./ Wt. Inc.	% Mort.
2-14-80/0	Oysters (H)*	23000			12.3	92	4/0.4	
	Water (H)*	240	10/10	3				
	Water (R)*	2	24/10	3				
2-24/10	Water (R)*	3	20/19	10				
	Oysters (R)				10.3	8	27/0.27	0
	Tray 1	20						
	Tray 2	40						
	Tray 3	20						
	Tray 4	20						
	Tray 5	20						
	Tray 6	20						
	Tray 7	20						
	Tray 8	20			10.3	8	27/0.27	
	Median	20						
	Oysters (C)	20			10.3	8	27/0.27	30

<sup>1</sup> Sample Code:

H = Harvest Area

\* = Average of Two Samples

R = Relaying Area

C = Onbottom Control Oysters

Tray = Phillips Coop

criteria for approved shellfish growing waters. Bottom layers of oysters in half-full baskets purged satisfactorily in 96 h, but at a slower rate than did single-layer oysters. Bottom layers of oysters in full baskets increased their bacterial content and failed to purge satisfactorily during the 96-h test periods. The failure was attributed to the higher weight of the overlying oysters and to the restriction of water flow over and through the stacked shellfish.

Quayle and Bernard (1976) demonstrated the practicality of cleansing containerized Pacific oysters (*Crassostrea gigas* Thunberg). Their galvanized, wire-mesh baskets (62 × 62 × 30 cm) were filled with approximately 55 kg of oysters and individually placed directly on the bottom. They found that environmental parameters exerted little influence on the cleansing process, with the oysters reaching equilibrium with ambient bacteriological conditions within 48 h. They suggested that such containers should provide an economical alternative to the traditional, single-layer, onbottom relaying with all of its inherent problems. They also suggested that containerized-relaying would be a useful preliminary step prior to depurating oysters with high initial MPN values.

Cook and Childers (1968) demonstrated that relaying is microbiologically feasible in Mississippi Sound. Under their experimental conditions, oysters purged to acceptable, safe bacteriological levels in eight days. Cook (1969) found no apparent differences in the elimination rates of indicator bacteria from onbottom oysters and oysters held 15 cm above the bottom.

#### Container Studies (Suspension-Relaying)

Some oysters exhibited a drop in the Condition Index

(CI) and Percent Spawnability (PS) (Tables 1, 3, and 5). Reductions in the CI and PS were also noticeable in data from other experiments. The spawning of oysters after transplantation into another habitat is not uncommon. During depuration experiments conducted at the Gulf Coast Research Laboratory (Bond et al. 1979), oysters regularly spawned after being transferred to depuration tanks during spawning season. Oysters relaid during the warmer months can, therefore, be expected to produce decreased volumes of shucked meats.

The Vexar<sup>®</sup> bags and Nestier<sup>®</sup> trays were smaller than the coops; and, therefore, they held less than a commercial quantity (1 sack) of oysters. The Vexar<sup>®</sup> material could be used only once because of abrasion of the fabric by the oysters' sharp shell margins. Relaid oysters can grow considerably (up to 1 cm) during 15 days, and the newly deposited shell material is razor-sharp. The Nestier<sup>®</sup> trays were unstable during suspension because most of the oysters accumulated on one side. Additional materials (e.g., support, flotation, and/or weight) would be required if these trays were used commercially.

The Piper coop proved advantageous for suspension-relaying. These coops could be stacked up to 11 high, with each containing 36.3 kg (80 lb) of shellfish, a plus for fork-lift handling, shipping, and/or storage. The Piper coops were also more suitable for stacking with better interlocking lid-to-bottom configurations.

Becker (1977) indicated that oysters in the middle of the container are the least likely to cleanse. The containers used during his studies were cuboidal in shape. The rectangular

shape of the coops in this study altered the internal volume configuration and increased the surface/volume ratio.

Suspension methods which use stacked containers and buoys are more practical than onbottom-relaying since they increase the use of the water column. Those methods would also eliminate the need for consistent bottom suitability, a particular problem in Mississippi Sound. Gunter and McGraw (1973) found that 59% of relaid, single oysters landed upside down when transplanted. Those oysters may thereby die from sediment-intake and gill-clogging problems. Similar problems were encountered during the 1977 commercial relaying program in Mississippi Sound. Lease holders found mortality rates of transplanted oysters of up to 99% (Mr. E. R. Gollott, Cap'n Gollott Seafood Co., Biloxi, MS, personal communication). Some areas were exposed to liquid mud flows from adjacent dredge and fill operations. The local commercial oyster industry is already equipped with necessary boat gear (i.e., booms, winches, etc.) to accommodate suspension-relaying. Buoys used for suspension should be detectable by radar.

#### *Onbottom-Relaying (Longline)*

Container burial was a factor in oyster cleansing during these experiments. The fully-loaded coops settled into the firm, mud-sand bottoms at Stations 2 and 3. This forced the termination of the second experiment after 13 days. Oysters from the bottom layer in coop 2 of that trial had FC values of approximately 270 MPN/100 g which were greater than the same oysters from coop 1 six days earlier. Mortalities of 20 to 25% exhibited by oysters in coops were also indicative of sediment problems.

More intra-container sedimentation was observed using this system. Recent literature (Smith et al. 1978) indicates that purged viruses may be deposited onto surrounding sediments. Ellender et al. (1980) states that sediments can contribute large numbers of viruses to the water column and possibly to feeding shellfish; therefore, direct bottom contact may be detrimental to consistent cleansing.

#### *Rack-Relaying*

Trials # 6 and 7 demonstrated that fluctuations can occur in the levels of indicator bacteria in oysters over time. This was apparent in oysters which exhibited increases in the FC values of approximately 2,300 MPN/100 g above the three-day value after seven days. During the second experiment FC MPN values also increased in oysters from 50% of the sampled trays, from day 7 to day 10 (Table 4). Reasons for those fluctuations were hard to determine without more extensive sampling of ambient water; however, fluctuations may have been caused (in part) by dead oysters and hook mussels (*Ischadium recurvum* [Rafinesquel]). Those putrefying bivalves may act as a reservoir for purged coliforms and other microbes, thus slowing the cleansing rate.

In every trial, the rack acted as an "artificial reef," with xanthid mud and stone crabs (*Menippe mercenaria* Say),

grass shrimp (*Paleomonetes* spp.), blue crabs (*Callinectes sapidus* Rathbun), and various fishes (e.g., Blennidae, Gobiidae, and Pomadasysidae) associated with the oysters. Sheepshead (*Archosargus probatocephalus* Walbaum) and small black drum (*Pogonias cromis* Linnaeus) were especially prevalent. Those associates may contribute to the removal of dead shellfish, thus helping to eliminate the problem of putrefying meats.

The oyster rack was the best method tested; however, it also was the most expensive (each rack costs approximately \$4,000). This system should be used in water depths greater than 3 m, to allow proper navigational clearance and to reduce recontamination by surface waters. The rack practically eliminates theft, because a fully loaded rack weighs approximately 3,600 kg (8,000 lbs) out-of-water. Stealing of individual coops from the rack is deterred by locking the vertical rows of containers in place with steel rods and hinged angle irons. The construction of the rack also permits the use of less suitable bottoms, because the timber runners and plywood sheet beneath the rack retard burial.

The Phillips tray was ideal for the rack system; hinged lids were not required, nor were stacking capabilities; and the perforated bottoms allowed adequate flow between oyster layers, for elimination of feces into the surrounding water.

A spacing distance of 30.5 m (100 ft) between each separate, daily relaying run is presently required by the FDA (Mr. Thomas Herrington, Seafood Specialist, Food and Drug Administration, Atlanta, GA, personal communication). Spacing requirements can be empirically estimated (Supan 1981) provided: (1) the oysters eliminate the initial bacterial load completely, and (2) the purged bacteria become evenly distributed in the water column. Assuming that commercial size oysters (70 to 109 mm) have an initial bacterial load of 23,000 MPN/100 g (the highest encountered in this study), a single rack's spacing requirement could be 7.22 m (23 ft). The FDA requirement represents a 4X safety factor. Because purged bacteria are located primarily in fecal ribbons and are not evenly dispersed in the water, spacing requirements could be further reduced. Smaller spacing distances can help reduce lease acreage necessary for relaying; however, such speculation must be substantiated.

#### ACKNOWLEDGMENTS

We acknowledge the Gulf Coast Research Laboratory (GCRL) for funding and the use of equipment, as well as the University of Southern Mississippi for its funding and cooperation. Thanks are extended to Piper Industries, of Jackson, MS, for donating the chicken coops and to E. R. Gollott for his support and the use of his equipment. David Cook of the GCRL Microbiology Section provided advice throughout the project and reviewed this manuscript. John Cirino and Ken Hase assisted in the field and Lucia O'Toole and Sheila Vanek typed this manuscript.

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## OPERATION OF AN OYSTER HATCHERY UTILIZING A BROWN WATER CULTURE TECHNIQUE

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**ABSTRACT** Raw bay water pumped from Biloxi Bay at Point Cadet in Biloxi, MS, and passed through a 5- $\mu$ m filter bag was found to be sufficiently nutritious to rear oyster larvae. Nitrate values of ambient water and the resulting phytoplankton as indicated by chlorophyll *a* determined over several years were found to be comparable to or exceed values for a cultured algal diet. Survival of larvae based on two years operation was 25%, and setting occurred within 8 to 13 days. Sexually mature oysters for spawning are naturally available 10 months out of the year. A facility costing under \$4,000.00 that can be operated by one person was constructed.

### INTRODUCTION

There are two classical types of shellfish hatcheries depending on how the larvae are fed. The "Milford" method relies on pure cultures of known algal species which are fed at controlled rates. The "Wells-Glancy" method utilizes bay water that is coarsely filtered by centrifugation, and fertilized to induce a bloom of naturally occurring algae. An alternate to the classical methods is the use of "raw" water coarsely filtered through fiber bags (General Analine Film Corp. [GAF] 5  $\mu$ m) as a food and culture media. This method, first reported by Hidu et al. (1969), was used by a commercial oysterman for several years on Chesapeake Bay (Frank Wilde, Chesapeake Oyster Culture, Inc., Shady Side, MD, personal communication). It permits the inexpensive operation of a hatchery by eliminating the expense of

rearing larval food. The Milford method is expensive and requires special facilities and additional technicians which comprise 30% of the hatchery operation (Krantz 1979). The Wells-Glancy method requires an expensive continuous centrifuge. In contrast, the use of filtered bay water requires only disposable filter bags, a pump, and associated plumbing, but is site-specific and dependent on ambient nutrients.

### MATERIALS AND METHODS

The culture facility consisted of a 3.9  $\times$  13-m (12  $\times$  40-ft) green house constructed of a double wall of polyethylene (Monsanto 602) stretched over polypropylene pipes anchored to the ground (Figure 1). The oyster larvae were reared in four cylindrical fiberglass tanks of 1,890-l (500-gal) capacity. Bay water was pumped by a 2-hp pump from a pier

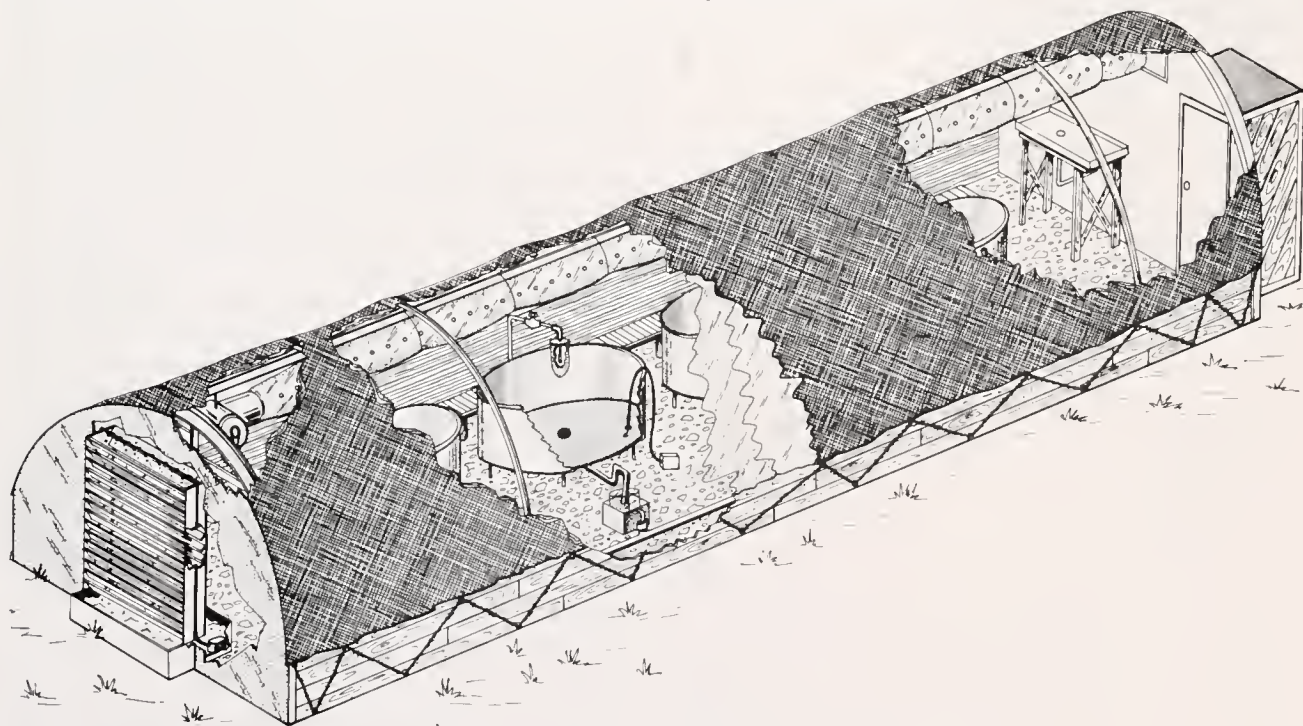


Figure 1. Polyethylene-covered greenhouse as used for an oyster hatchery.

extending 46 m (150 ft) into Biloxi Bay and passed through a 5- $\mu$ m filter bag into the culture tanks. The water was not fertilized or aged. The tanks were initially stocked with  $20 \times 10^6$  larvae, and the water was completely changed three times weekly. The tanks were drained through 2.54-cm (1-in) pipes into a sieve box which collected the larvae as the water flowed to a waste drain. At each change, larvae were concentrated into 10 l of water, stirred with a plunger plate, and a 1-ml sample was withdrawn and the total number of larvae was estimated. Tanks, drains, and air lines were alternated with each water change. Tanks were hosed with fresh water, scrubbed, and allowed to air dry between changes. Aeration was provided in each tank by a single air stone from an aquarium vibrator pump.

Nitrate was determined by cadmium reduction and read colorimetrically. Chlorophyll *a* was extracted in acetone and read spectrophotometrically (Strickland and Parsons 1968). Gonadal condition was determined microscopically by examining smears from 40 oysters collected monthly over a 22-month period.

## RESULTS

Oysters (*Crassostrea virginica*) in Mississippi are potentially capable of spawning 8 to 10 months a year. They ripen during March when the temperature first exceeds 20°C. Spawnable stocks are available through October and into December in some years depending upon their location in Mississippi Sound. Oysters are generally difficult to spawn during July when temperatures exceed 30°C (Table 1); however, spawning may be accomplished in the warm months by storing the oysters out of water in a cool place for a few days.

TABLE 1.

Percent of sexually developed oysters based on mean of samples collected from four reefs over a 22-month period.

Month	Percent Developed
January	0.0
February	0.0
March	8.5
April	72.5
May	88.0
June	92.8
July	78.8
August	93.8
September	85.0
October	20.0
November	7.5
December	7.5

The levels of nitrate in the bay were more than sufficient to sustain phytoplankton growth. The level of nitrate in Provasoli's Asp 2 media for phytoplankton culture was 8 mg/l (Fogg 1966). That value or higher of nitrate was

normally present in ambient water (Figure 2) with values exceeding 30 mg/l noted at various times.

The phytoplankton content of the ambient water as indicated by the chlorophyll *a* content was also more than sufficient for larval culture. A mixture of *Monochrysis lutheri* and *Isochrysis galbana*, the traditional diet for oyster larvae, at a concentration of 250,000 cell/ml, had a 0.5-mg/m<sup>3</sup> chlorophyll *a* content. That value was normally exceeded in samples of ambient bay water, and values as high as 4.3 mg/m<sup>3</sup> were recorded (Figure 3). No attempt was made to identify the dominant algal species but the phytoplankton imparted a brown color to the water, hence the term "brown water" culture.

Survival of the larvae through metamorphosis was 25% when the larvae were stocked at 10/ml and was based on 11 broods reared over a 2-year period. These data include two broods terminated before setting. Survival in some broods was as high as 60%. Under experimental conditions when stocking densities were reduced to 5 larvae/ml, survival as high as 81% was noted. Time from spawning to first setting ranged from 7 to 13 days.

The greenhouse was constructed in 1977 at a cost of \$500.00. The four fiberglass tanks cost \$500.00 each when purchased. The facility with pumps, plumbing, and wiring cost under \$3,000.00 making it "expendable" under the hurricane conditions that occur along the Gulf coast. It is possible to equip the facility for an additional \$1,000.00.

## DISCUSSION

Historically, the Gulf of Mexico has been an area of high oyster production with an abundant spat set. For this reason, the development of oyster hatcheries was delayed until recent years. The operation of an oyster hatchery in an area of high natural production has several advantages as pointed out by Ogle (1980). The availability of spawning stocks of oysters over most of the year, the lack of a need to rear food for the larvae, and the use of a low-cost facility permits the inexpensive production of larvae.

Although oysters in Biloxi Bay have the potential to spawn 8 to 10 months of the year, natural spawning at this site occurs during only 5 to 7 months. Contrary to what one would expect from the literature (Hopkins 1954), setting occurs during only 1 to 4 months of the year (Ogle 1979a), whereas an oyster hatchery can operate throughout most of the year. The lack of spawnable oysters during the winter prevents year-round operation of the facility as an oyster hatchery rather than the absence of sufficient food in the water. This technique has been successfully used to rear copepod larvae during winter months (Ogle 1979b). Attempts to condition oysters from the Gulf of Mexico to spawn out of season have not been successful. It is possible that a cold-water species of oyster such as *Ostrea* could be cultured during the winter.

Failure of oyster sets have been reported in Texas during 1976–1977 (Sammy M. Ray, Texas A&M at Galveston, TX,

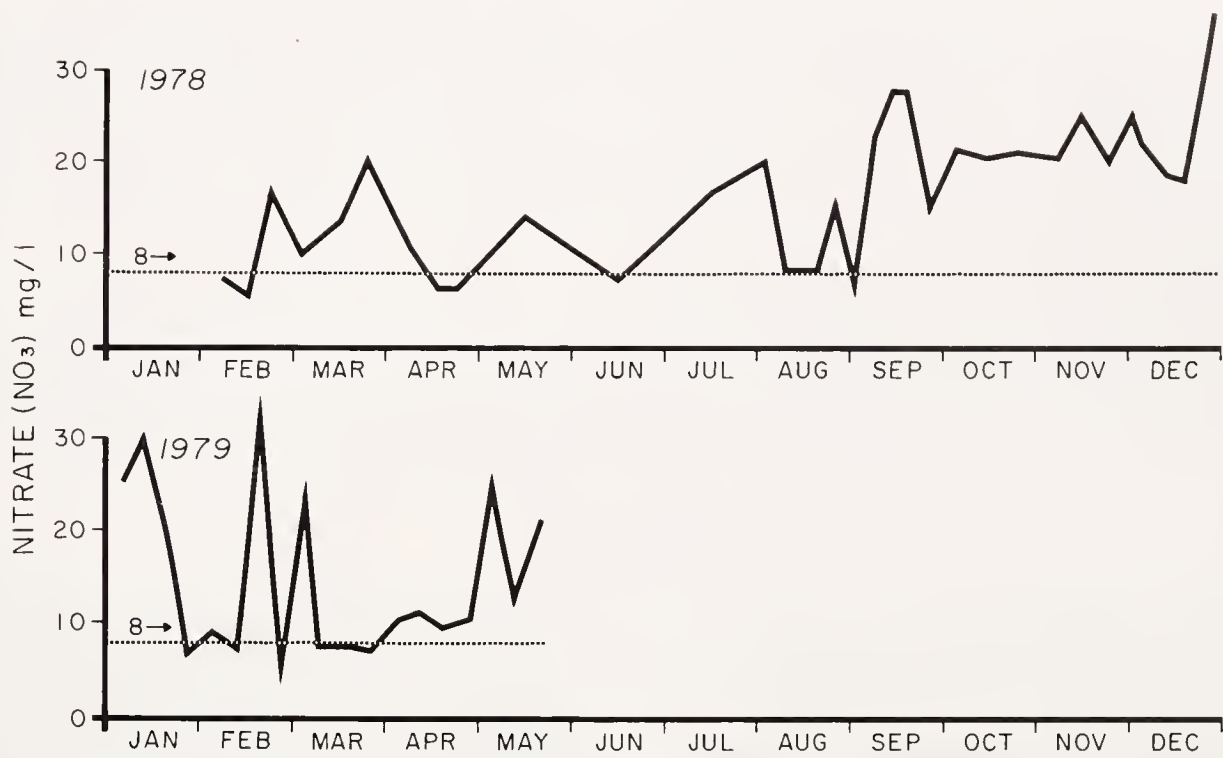


Figure 2. Nitrate concentration ( $\text{mg/l}$ ) determined weekly for ambient Biloxi Bay water during part of 1978 and 1979.

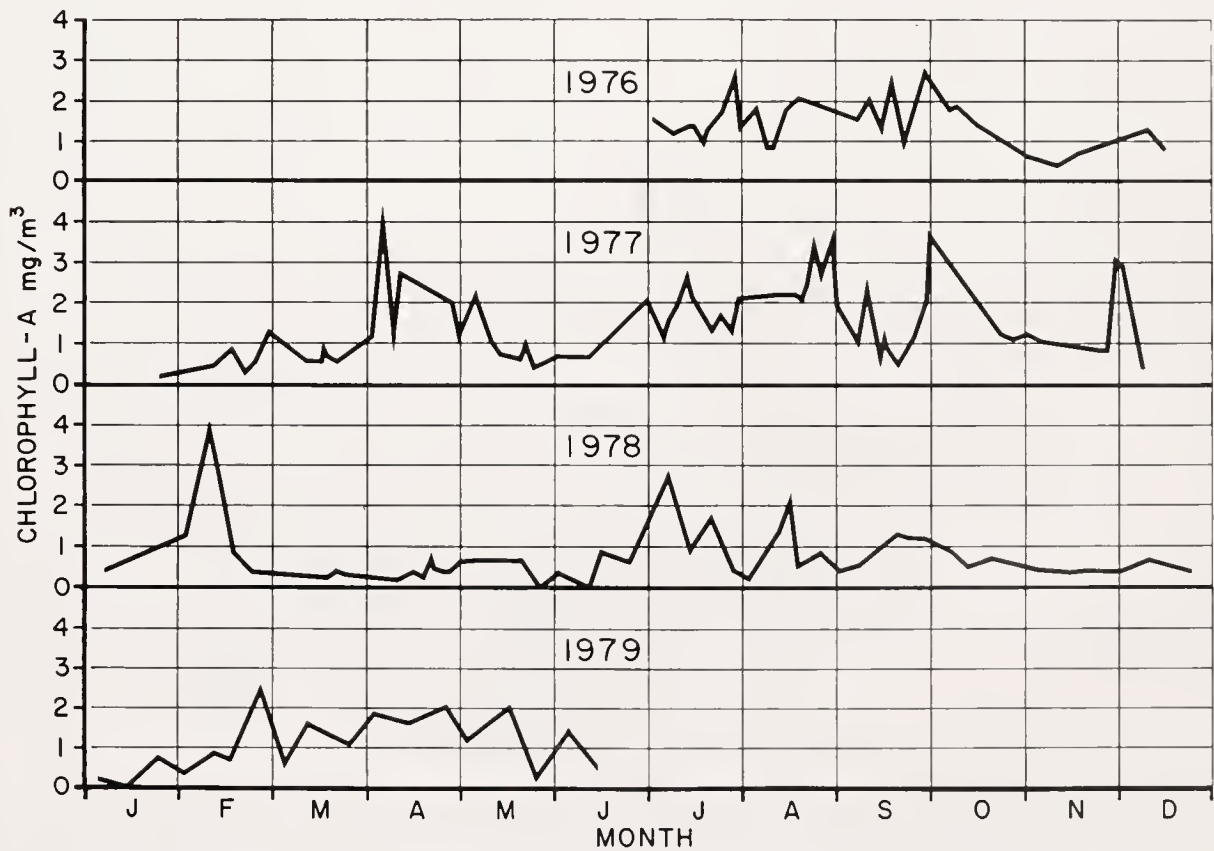


Figure 3. Chlorophyll *a* concentration ( $\text{mg/m}^3$ ) of ambient Biloxi Bay water over the period July 1976 to June 1979.

personal communication) and Louisiana during 1977–1978 (Charles N. Dugas, St. Amant Marine Laboratory, Grand Terre Island, LA, personal communication). Flooding in Mississippi during 1978 and 1979 caused set failures and depletion of oysters in much of Mississippi Sound. If this trend continues over the next few years, hatcheries will not only be feasible but may become a necessity. In the past, shell plantings for rehabilitation of reefs have not always succeeded because of the lack of coordination between shell planting times and the occurrence of larvae in the water. A hatchery can guarantee a set; however, the problems associated with moving the large volume of shells required for setting the larvae and maintaining a “cottage level” industry will have to be evaluated. The recent development of oyster farms in Mississippi lends promise that a

cooperative venture may be formed between this state-funded hatchery and private oyster farmers. This nonclassical approach to the operation of an oyster hatchery lends itself to individual oyster farmers or a “cottage level” industry. While the method is site-dependent, it should be the first method attempted by all persons desiring to establish an oyster hatchery.

#### ACKNOWLEDGMENTS

Appreciation is expressed to those who have worked “at the hatchery” especially Kathleen Beaugez, John Supan, Rich Sherrard, and the Gulf Coast Research Laboratory mariculture class of 1980. Beryl Heard prepared Figure 1, and Lucia O'Toole typed the manuscript.

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## ALLOZYME VARIATION IN THREE NONSIBLING *OSTREA* SPECIES

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**ABSTRACT** Estimated levels of genic variation and genetic similarity are reported for three nonsibling species of *Ostrea*. These estimates are based on an examination of 25 to 29 structural loci. The proportion of polymorphic loci per species was estimated as 0.276, 0.370, and 0.520 for *O. edulis*, *O. lurida*, and *O. permollis*, respectively. The corresponding observed heterozygosities per individual were estimated as 9, 16, and 15%. The genetic similarities and distances between the three nonsibling species were computed. Also, a pairwise comparison of loci was made between species which indicated that approximately 17% of the loci studied were genetically identical while 55% had no genetic similarity. The mean genetic identity across all loci among the three species was estimated as 0.245. Finally, there seemed to be a correlation between the dispersal time of planktonic oyster larvae and the levels of genetic variation found within the three species.

### INTRODUCTION

Several recent reports describe the levels of genetic variation among euryhaline and oviparous oyster species of the genera *Crassostrea* and *Saccostrea* (Schaal and Anderson 1974; Buroker et al. 1975, 1979a,b; Singh and Zouros 1978; Zouros et al. 1980). In contrast there are no reports containing overall estimates of genetic variation in the stenohaline, larviparous *Ostrea* species. Studies of protein variation, using methods of gel electrophoresis, indicate the presence of allozyme polymorphism in *O. edulis* (Wilkins and Mathers 1973, 1974) and *O. lurida* (Johnson et al. 1972); however, those studies were limited to one or two protein variants and by no means constituted a survey of genic variation and genetic similarity among *Ostrea* species. Here I provide such a survey for *O. edulis*, *O. lurida*, and *O. permollis*.

The European oyster *O. edulis* (Linnaeus), which occurs naturally in bays and estuaries from low tide to a depth of 80 m, has a wide geographical distribution along the Atlantic, North Sea, and Mediterranean coasts of Europe. Throughout its range its natural breeding population has steadily declined because of overfishing, pollution, and habitat destruction. Efforts have been made through aquaculture and management programs to maintain this oyster fishery in Europe (Yonge 1960).

The Olympic oyster *O. lurida* (Carpenter), which occurs naturally in bays and lagoons from low tide to a depth of 71 m, has a wide distribution along the Pacific coastlines of Canada, Mexico, and the United States (Hertlein 1959). Throughout its range it has also experienced a steady decline in natural breeding populations primarily because of overfishing, pollution, and competition for resources with the Japanese oyster *Crassostrea gigas* (Thunberg), which was

introduced into the United States approximately 80 years ago.

The sponge-oyster *O. permollis* (Sowerby) occurs in the northeastern Gulf of Mexico and along the coast of North Carolina (Forbes 1964). This oyster is commensal in the sponge *Stelletta grubii* (Schmidt) where adults live either on the surface of the sponge or partly embedded. Because *O. permollis* is host specific to one species of sponge, its distribution is limited to that of its host. Sponges containing *O. permollis* occur subtidally on rock, sand, and sea-grass bottoms to depths of 154 m.

### MATERIALS AND METHODS

Specimens of *O. edulis* were obtained from natural stock at Boothbay Harbor, Maine. This stock originated from the Oosterschelde in Holland in the 1940's (Welch 1963; Peter Korringa, personal communication). Samples of *O. lurida* were collected from Rocky Bay, Hood Canal, Washington. Samples of *O. permollis* were obtained from Alligator Harbor, Franklin County, Florida.

The methods of protein electrophoresis and biochemical genetic techniques used in this study have been described previously (Buroker et al. 1975, 1979a). Oyster samples were frozen immediately ( $-40^{\circ}\text{C}$ ) upon arrival at the laboratory and remained frozen until the time of electrophoretic analysis. Eighteen protein staining procedures were used in this investigation which reflected from 25 to 29 structural loci in these *Ostrea* species. The genetic loci were sampled on the basis of available staining procedures and clarity of banding. The selection of loci was without bias to levels of protein variability within these species.

### RESULTS

#### Genic Variation

The allelic variation is tabulated for the three *Ostrea* species in Table 1. The results indicate for each species the enzyme (protein) synthesizing loci (e.g., AcP-1, AcP-2, etc.).

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the number of alleles ( $n$ ) sampled at each locus, the relative allelic mobilities (e.g., 104, 100, 96, etc.) at each locus measured from an internal standard on the starch-gel, the relative allelic frequencies for each locus, the observed heterozygosity ( $H$ ) per locus, and the deviation of observed from Hardy-Winberg expected heterozygous genotypes [ $D = (H_o - H_e)/H_e$ ]. A positive  $D$  indicates an excess of observed heterozygous genotypes while a negative value indicates a deficiency of heterozygous genotypes. Table 1 can be visually analyzed in the following manner. The 100-allele frequencies for the Adk-1 locus can be compared among the three *Ostrea* species by examining the values to the right of the Adk-1 allele under each species heading. The level of Adk-1 variation can be compared by examining the observed heterozygosity ( $H$ ) under each species heading. Table 1 indicates that considerable differences in allele frequencies and observed heterozygosity exist among the three *Ostrea* species.

The number of loci studied, mean number of genes sampled per locus, and level of genetic variation (recorded as population polymorphism and individual heterozygosities) are presented in Table 2. The polymorphism value (i.e., number of polymorphic loci divided by total number of loci observed within a population) gives an estimate of the number of loci which exhibit genic variation from a random sample of the structural genes in a population. Thus, the amount of polymorphism is an estimate of the genetic variation at the population level. These values (Table 2) were 0.276, 0.370, and 0.520 for *O. edulis*, *O. lurida*, and *O. permollis*, respectively. Another estimate of the amount of genetic variation within a population is its heterozygosity, i.e., an estimate of genic variation at the individual level. For example, if a certain species exhibits a heterozygosity of 10%, it means that an individual of that species will be, on the average, heterozygous at 10% of its structural loci. These values (Table 2) are reported as the observed frequency based on Hardy-Winberg equilibrium. The observed individual heterozygosities were 0.088, 0.156, and 0.148 for *O. edulis*, *O. lurida*, and *O. permollis*, respectively.

#### Genetic Similarity and Distance

Genetic similarity and distance can be estimated from the number of alleles that any two populations of species have in common (Nei 1972). When the genetic similarity between two sampling groups is 1.00, the groups are said to be genetically identical. In this instance the genetic distance would be zero by definition (Nei 1972). Such estimates were computed between the three *Ostrea* species (Table 3). These data indicate that the three nonsibling species are genetically similar to 15 to 29% of their structural loci.

#### Pairwise Comparison of Loci

A pairwise comparison of loci was conducted among the three *Ostrea* species. The results of this comparison

were plotted as a histogram (Figure 1). The mean genetic identity ( $I$ ) for all loci compared among the three species was  $0.245 \pm 0.068$ . If Nei's (1972) measure for genetic distance [ $D = -\log I$ ] is computed to give an estimate of the accumulated number of codon differences per locus, a value of  $1.406 \pm 0.188$  is obtained. This indicates that, on the average, more than one electrophoretically detectable allelic substitution per locus has occurred between these nonsibling *Ostrea* species.

#### DISCUSSION

Because the founder effect (i.e., founders of a new population contain only a small fraction of the total genetic variation of the parental population [Mayr 1963]) can significantly alter genic variation and levels of genetic variation in natural populations of biota (White 1978), this effect should be considered in the analysis of *O. edulis*.

Recalling that spat of *O. edulis* were originally transported from Holland to the United States in the 1940's, it is possible that the founder effect and genetic drift could have lead to changes in allele frequencies since the time the natural stock was established in Boothbay Harbor, Maine. Wilkins and Mathers (1973) reported allele frequencies for esterase (Est) and phosphoglucose isomerase (Pgi) variation in *O. edulis*. They sampled three populations in Ireland and one in Norway. The Norwegian population was more similar to the parental population of the Boothbay Harbor samples from Holland than the Irish populations.

The results of Wilkins and Mathers (1973) for their four populations and my Boothbay Harbor samples were compared with respect to Est and Pgi allozyme variation. Wilkins and Mathers (1973) reported three zones of esterase activity (i.e., EsF, EsM, and cathodal esterase) which correspond to my Est-1, Est-2, and Est-3 structural loci, respectively (Table 1). In the Norwegian population they reported the common allele frequencies for EsF as 1.00, EsM as 0.656, and no results for cathodal esterase. From Table 1, it can be seen that the common allele frequencies of the Boothbay Harbor samples of *O. edulis* for Est-1 is 1.00 and for Est-2, 0.642. They also gave the common allele frequency of the Pgi locus as 0.992 for the Norwegian population and, as shown in Table 1, the frequency of this allele for the Boothbay Harbor samples is 0.988.

These results indicate minimal allele frequency difference among the three structural loci for the Norwegian population and the founder population from Holland. Although this is not a conclusive test for the congruence of gene frequencies between the two populations, it does suggest that the natural stock maintained at Boothbay Harbor, Maine, has not changed much from at least one population along the northern European coastline.

Johnson et al. (1972) reported allozyme variation for aspartate aminotransferase and muscle protein in *O. lurida*. Their results do not coincide with the survey of protein

TABLE 1.

Genetic variation in three nonsibling species of *Ostrea*: *O. edulis* (1), *O. lurida* (2), and *O. permollis* (3).

(RM: relative allelic mobility; n: number of genes sampled; H: observed heterozygosity;

—: no data; D:  $[(H_o - H_e)/H_e]$ .)

Locus	Allele RM	(1)	(2)	(3)	Locus	Allele RM	(1)	(2)	(3)
AcP-1	n	160	160	----	Aat-1	100	1.000	1.000	0.867
	104	1.000	0.000	----		96	0.000	0.000	0.067
	100	0.000	1.000	----		H	0.000	0.000	0.240
	H	0.000	0.000	----		D	0.000	0.000	0.111
	D	0.000	0.000	----					
AcP-2	n	160	160	----	Aat-2	n	160	200	----
	110	1.000	0.000	----		100	1.000	0.065	----
	100	0.000	1.000	----		95	0.000	0.935	----
	H	0.000	0.000	----		H	0.000	0.130	----
	D	0.000	0.000	----		D	0.000	0.066	----
AcP-3	n	160	80	----	Est-1	n	160	----	30
	110	0.000	0.925	----		104	1.000	----	0.033
	105	1.000	0.075	----		100	0.000	----	0.900
	H	0.000	0.100	----		96	0.000	----	0.067
	D	0.000	-0.286	----		H	0.000	----	0.184
						D	0.000	----	0.084
AdK-1	n	160	152	28	Est-2	n	154	160	30
	108	0.000	0.000	0.250		104	0.247	1.000	0.000
	104	1.000	0.000	0.643		102	0.032	0.000	0.000
	100	0.000	0.000	0.107		100	0.078	0.000	1.000
	98	0.000	0.434	0.000		98	0.642	0.000	0.000
	96	0.000	0.566	0.000		H	0.506	0.000	0.000
	H	0.000	0.474	0.500		D	-0.023	0.000	0.000
	D	0.000	-0.035	-0.025					
AdK-2	n	----	----	28	Est-3	n	154	148	30
	98	----	----	1.000		108	0.000	0.189	0.067
	H	----	----	0.000		104	0.000	0.554	0.767
	D	----	----	0.000		100	0.435	0.257	0.167
						96	0.208	0.000	0.000
Ald	n	160	160	30		92	0.357	0.000	0.000
	104	1.000	0.000	1.000		H	0.610	0.608	0.467
	96	0.000	1.000	0.000		D	-0.047	0.027	0.228
	H	0.000	0.000	0.000					
	D	0.000	0.000	0.000	G3pdh	n	----	160	26
						106	----	0.000	0.154
Ap-1	n	152	158	30		100	----	0.000	0.846
	106	0.000	0.000	0.033		90	----	1.000	0.000
	103	0.000	0.000	0.067		H	----	0.000	0.308
	100	0.803	0.000	0.900		D	----	0.000	0.182
	97	0.197	0.019	0.000	ldh-1	n	158	160	30
	94	0.000	0.797	0.000		104	0.911	0.000	0.000
	91	0.000	0.184	0.000		100	0.089	0.000	0.967
	H	0.368	0.291	0.200		98	0.000	1.000	0.033
	D	0.163	-0.119	0.084		H	0.177	0.000	0.067
						D	0.094	0.000	0.034
Ap-2	n	160	160	30	ldh-2	n	160	160	30
	100	1.000	1.000	0.000		104	0.000	1.000	1.000
	92	0.000	0.000	1.000		100	1.000	0.000	0.000
	H	0.000	0.000	0.000		H	0.000	0.000	0.000
	D	0.000	0.000	0.000		D	0.000	0.000	0.000
Aat-1	n	160	160	30					
	104	0.000	0.000	0.067					

TABLE 1. (Continued)

Genetic variation in three nonsibling species of *Ostrea*: *O. edulis* (1), *O. lurida* (2), and *O. permollis* (3).  
 (RM: relative allelic mobility; n: number of genes sampled; H: observed heterozygosity;  
 -: no data; D:  $[(H_o - H_e)/H_e]$ .)

Locus	Allele RM	(1)	(2)	(3)	Locus	Allele RM	(1)	(2)	(3)
Lap-1	n	160	----	30	Mp-2	n	160	200	----
	108	1.000	----	0.000		110	0.000	1.000	----
	92	0.000	----	0.067		100	1.000	0.000	----
	89	0.000	----	0.933		H	0.000	0.000	----
	H	0.000	----	0.133		D	0.000	0.000	----
	D	0.000	----	0.071					
Lap-2	n	160	160	28	6Pgdh	n	160	72	26
	110	1.000	0.000	0.000		106	0.000	0.000	0.038
	106	0.000	0.000	0.893		103	0.000	0.431	0.038
	104	0.000	0.000	0.107		100	1.000	0.569	0.500
	102	0.000	0.506	0.000		97	0.000	0.000	0.423
	100	0.000	0.163	0.000		H	0.000	0.306	0.462
	98	0.000	0.331	0.000		D	0.000	-0.375	-0.188
	H	0.000	0.525	0.214	Pgi	n	160	200	24
	D	0.000	-0.136	0.120		108	0.000	0.630	0.000
Mdh-1	n	160	160	30		103	0.000	0.370	0.000
	120	0.000	0.000	1.000		97	0.988	0.000	0.000
	112	1.000	0.000	0.000		94	0.000	0.000	0.333
	100	0.000	1.000	0.000		90	0.013	0.000	0.667
	H	0.000	0.000	0.000		H	0.025	0.500	0.333
	D	0.000	0.000	0.000		D	0.010	0.073	-0.250
Mdh-2	n	160	160	30	Pgm-1	n	160	200	30
	105	0.094	1.000	1.000		104	0.000	0.075	0.000
	100	0.906	0.000	0.000		102	0.000	0.565	0.100
	H	0.188	0.000	0.000		100	0.588	0.360	0.600
	D	0.103	0.000	0.000		98	0.381	0.000	0.200
						96	0.031	0.000	0.100
Me	n	160	160	30		H	0.463	0.540	0.600
	104	0.000	0.000	1.000		D	-0.091	-0.011	0.034
	100	0.000	1.000	0.000	Pgm-2	n	160	----	30
	98	1.000	0.000	0.000		100	1.000	----	1.000
	H	0.000	0.000	0.000		H	0.000	----	0.000
	D	0.000	0.000	0.000		D	0.000	----	0.000
Mpi-2	n	160	160	30	TO-1	n	160	200	----
	102	0.000	0.000	0.200		110	1.000	0.000	----
	100	0.000	0.000	0.567		100	0.000	1.000	----
	98	0.000	0.000	0.133		H	0.000	0.000	----
	96	0.881	0.888	0.100	TO-2	D	0.000	0.000	----
	92	0.094	0.000	0.000		n	160	200	30
	88	0.025	0.113	0.000		100	1.000	0.000	0.000
	H	0.238	0.200	0.667		98	0.000	1.000	0.000
	D	0.111	0.001	0.901		90	0.000	0.000	1.000
Mp-1	n	160	200	30		H	0.000	0.000	0.000
	108	0.000	0.000	1.000		D	0.000	0.000	0.000
	102	1.000	0.000	0.000	Xdh	n	160	160	30
	100	0.000	1.000	0.000		104	0.000	1.000	0.000
	H	0.000	0.000	0.000		102	1.000	0.000	0.000
	D	0.000	0.000	0.000		98	0.000	0.000	1.000
						H	0.000	0.000	0.000
						D	0.000	0.000	0.000

TABLE 2.

Summary of genetic variation in natural populations of nonsibling species of *Ostrea*. A locus is considered polymorphic when the frequency of the most common allele is  $\leq 0.99$ . The heterozygous values are based on the observed number and Hardy-Weinberg expected number of heterozygotes per individual.

Species	<i>O. edulis</i>	<i>O. lurida</i>	<i>O. permollis</i>
Number of loci studied	29	27	25
Mean number of genes sampled per locus $\pm$ SD	159 $\pm$ 2	163 $\pm$ 31	29 $\pm$ 2
Polymorphic loci per population	0.276	0.370	0.520
Heterozygous loci per individual			
observed	0.088 $\pm$ 0.004	0.156 $\pm$ 0.005	0.148 $\pm$ 0.013
expected	0.087 $\pm$ 0.004	0.162 $\pm$ 0.006	0.168 $\pm$ 0.014

TABLE 3.

Estimates of genetic similarity and distance between three nonsibling species of *Ostrea*. Values above the diagonal are estimates of genetic similarity, those below are estimates of genetic distance (Nei 1972).

	(1)	(2)	(3)
(1) <i>O. edulis</i>	-----	0.1488	0.2884
(2) <i>O. lurida</i>	1.9054	-----	0.2613
(3) <i>O. permollis</i>	1.2434	1.3421	-----

variation conducted here (Table 1). These discrepancies have been discussed elsewhere (Buroker et al. 1975).

Species of the genus *Ostrea* differ from those of the genera *Crassostrea* and *Saccostrea* in bio-ecological, morphological, and physiological characteristics (cf., Ahmed 1975). *Crassostrea* and *Saccostrea* species are considered euryhaline whereas *Ostrea* species are considered stenohaline (Yonge 1960). *Crassostrea* and *Saccostrea* species are oviparous whereas *Ostrea* species are larviparous. Oviparous species have a promyal chamber while larviparous species do not. Yonge (1960) and Galtsoff (1964) suggested that this chamber allows oviparous species to occupy the more turbid euryhaline conditions of estuaries. Although there is no difference in chromosome number ( $2N = 20$ ) and arm number (40) reported between oyster species of the *Crassostrea*, *Ostrea*, and *Saccostrea* genera (Ahmed and Sparks 1967, Longwell et al. 1967, Menzel 1968a,b), the *Ostrea* species contain "lampbrush" chromosomes due to loops from the chromosome axis (Ahmed 1975). Ahmed (1975) considered the loop formation as a manifestation of gene activation for the synthesis of RNA which, in turn, is required for the production of a greater quantity of yolk material in the oocytes of *Ostrea* species than that required for *Crassostrea* and *Saccostrea* species. In all reported cases the eggs of larviparous species have been found to be twice as large or larger than those of the oviparous species.

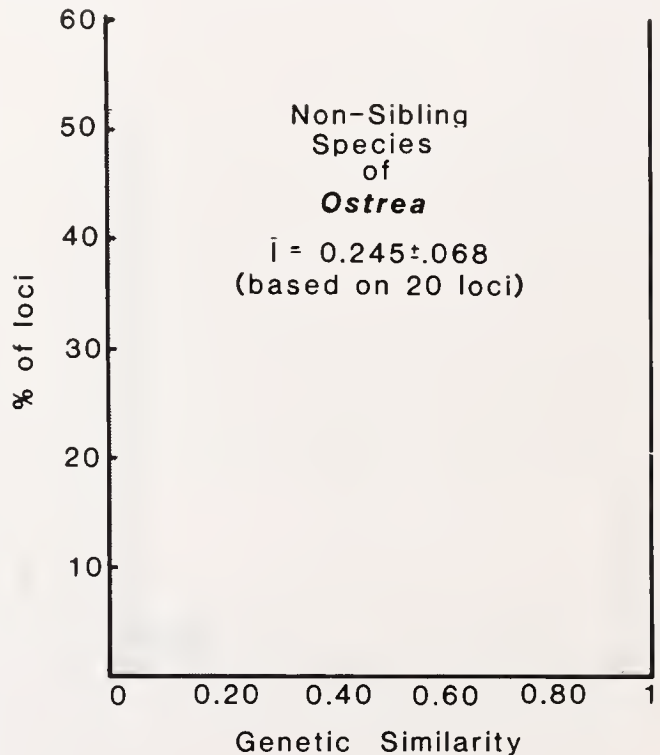


Figure 1. Distribution of loci with respect to genetic similarity. Pair-wise comparison of homologous loci between three nonsibling species of *Ostrea* (*O. edulis*, *O. lurida*, and *O. permollis*).  $\bar{I}$  = mean genetic similarity  $\pm$  standard error.

In light of these differences among oviparous and larviparous oyster species, the three *Ostrea* species that were studied have levels of genetic variation consistent with those found for *Crassostrea* and *Saccostrea* species (Buroker et al. 1979a,b). Although the *Ostrea* species are larviparous, they do have planktonic larval stages of development which in *O. edulis* ranges from 6 to 14 days (Cole and Knight-Jones 1939, Korringa 1941, Waugh 1957); in *O. lurida* it ranges from 10 to 23 days (Davis 1949); and in *O. permollis* it ranges from 30 to 33 days (Forbes 1962, 1964). This provides the means for dispersing larvae and allows ample

opportunity for gene flow to occur among populations within the three species. Since gene flow is an important evolutionary vector for transmitting new variants to surrounding demes (Mayr 1963, Dobzhansky 1970), it provides the means for maintaining high levels of genetic variation in marine pelecypod species which disperse larvae. An interesting relationship can be seen between the length of planktonic larval phase and levels of genetic variation (Table 2). *Ostrea edulis* which has the shortest planktonic larval phase also has the lowest level of genetic variation (i.e., polymorphic loci per population and observed heterozygosity). On the other hand, *O. lurida* and *O. permollis*, which have longer planktonic larval phases, have higher levels of genetic variation. A Spearman rank correlation test revealed a significantly similar relationship between planktonic larval phase and the proportion of polymorphic loci per population, but no correlation was found between planktonic larval phase and individual heterozygosity.

With the discovery that the rate of substitution in many structural genes over time is relatively constant regardless of differential rates of morphological evolution (Kimura 1969), a molecular clock became available that could be used in estimating cladistic events over geological time (Wilson et al. 1977). With this in mind a comparison can be made between the origin of a genus via the fossil record and the average genetic identity among living nonsibling species within the genus. Buroker et al. (1979b) found that the *Saccostrea* genus exhibited a higher degree of genetic similarity (i.e.,  $I = 0.736$ ) between nonsibling species than does the *Crassostrea* genus (i.e.,  $I = 0.356$ ) which indicated that the *Sac-*

*costrea* genus is a relatively new genus when compared with the *Crassostrea* genus. This conclusion coincides well with the fossil record where Stenzel (1971) has placed the origin of the *Saccostrea* genus in the Miocene period and the *Crassostrea* genus in the Cretaceous period. Because Stenzel (1971) also placed the origin of the *Ostrea* genus in the Cretaceous period, it would be expected that the genetic similarity between nonsibling species of *Ostrea* should have a relatively low value which it does ( $I = 0.245$ ).

In summary, *O. edulis*, *O. lurida*, and *O. permollis* maintain high levels of genetic variation among natural populations and have levels of genetic variation consistent with those found in species of *Crassostrea* and *Saccostrea*. In addition, the length of dispersal time of planktonic oyster larvae of these *Ostrea* species appeared to be related to the proportion of polymorphic structural loci maintained in a population.

#### ACKNOWLEDGMENTS

I express my gratitude to Herb Hidu and R. Winston Menzel for supplying oyster samples and to the Maryland and Washington State Sea Grant programs for partial support. In addition, I thank the College of Fisheries, University of Washington, and Center for Environmental and Estuarine Studies, University of Maryland, for their support in this study. Also, I express my gratitude to the Charles and Johanna Busch Foundation, Bureau of Biological Research, Rutgers, The State University of New Jersey, for partial support.

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## AN EVALUATION OF “SPAWNER TRANSPLANTS” AS A MANAGEMENT TOOL IN LONG ISLAND’S HARD CLAM FISHERY

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**ABSTRACT** A traditional management practice in New York’s hard clam (*Mercenaria mercenaria*) fishery has been to transplant adult clams from cooler northern waters to the relatively warmer waters of Great South Bay. It is believed that such spawner transplants increase the length of time that clam larvae are present in the bay and, thereby, enhance the probability that at least some of the larvae will encounter favorable conditions for survival and settlement. Histological analysis of the gametogenic cycle of native and transplanted clams showed that two critical assumptions were unsound: (1) that spawning by the native clams is defined and predictable, and (2) that the transplanted clams spawn after the native clams have ceased spawning. Other considerations, including the scale of the transplant projects relative to the natural stocks, suggest that these programs are unlikely to significantly increase recruitment in Great South Bay.

### INTRODUCTION

Great South Bay covers approximately 24,282 ha (60,000 acres) on the southern shore of Long Island, NY, and is the single largest producer of hard clams (*Mercenaria mercenaria* [Linné]) in the world. In 1980, reported landings from the public commercial fishery in Great South Bay were in excess of 14,400 m<sup>3</sup> (400,000 bu) with a dockside value of nearly \$20 million (Fred Blossom, National Marine Fisheries Service, Patchogue, NY, personal communication).

A number of management practices are applied to the hard clam fishery of Great South Bay. Most important among these are gear restrictions, a minimum legal size of one-inch total thickness (Bricelj and Malouf 1981), planting of seed clams (reviewed by McHugh 1981), and a practice known locally as “spawner transplants.” None of these practices has ever been thoroughly evaluated.

The origins of transplanting spawning stock to Great South Bay are not documented, but they appear to have started in this area in about 1963 (Hendrickson, NY Dep. Environ. Conserv., personal communication). The practice involves harvesting adult clams during June and July from the relatively cool waters of Long Island Sound or Cape Cod. The clams are subsequently released into the warmer waters of Great South Bay in the hope that they will then spawn. The rationale for the practice is based primarily on the belief that the reproductive success of hard clams is largely dependent on the chance co-occurrence of clam larvae and suitable environmental conditions. It would follow, then, that the longer that larvae from various sources are present in the bay the greater the chances are that at least some will encounter favorable conditions. The timing of spawner transplantations

are crucial to their success, and in that sense they differ conceptually from the simple movement of adult clams to increase the size of spawning stocks. Moreover, because the actual process of transplanting is usually carried out by a private contractor, the timing of the transplant must be established well in advance.

Loosanoff (1937) noted that clams from colder waters spawn somewhat later than those from warmer shallow waters. This observation and subsequent studies of gametogenesis and spawning by hard clams from other locations (Porter 1964, Keck et al. 1975, Eversole et al. 1980) support the view that it is technically possible to obtain and transplant clams whose spawning cycle is not synchronized with the Great South Bay populations.

Because of their low purchase price and relatively high fecundity, large “chowder” clams are generally used as transplants. There are no special harvest restrictions placed on the clams after they are transplanted, but their relatively low market value tends to reduce harvest pressure on them. Efforts are made to position transplanted clams so that larvae produced by them “seed” specific areas, although little is presently known about circulation and larval dispersal in Great South Bay.

The spawner transplant concept involves a great many assumptions. Some of these assumptions relate to the survival of larvae and to the relationships (if any) between the number of metamorphosing larvae and the number of clams eventually recruited to the fishery. Other, more readily tested assumptions relate to the gametogenesis and spawning of the native and transplanted clams. It is these assumptions that were the object of this study. Specifically, our objectives

were to histologically compare gametogenesis and spawning in native Great South Bay clams with that of a transplanted population. In this way the following assumptions were tested:

1. that native clams have a defined and predictable spawning period;
2. that native clams complete spawning prior to spawning of transplanted clams;
3. that transplanted clams do not spawn prior to being moved to Great South Bay; and
4. that transplanted clams do, in fact, spawn after being harvested, moved, and released into the bay.

#### MATERIALS AND METHODS

Hard clams were obtained on 14 June 1978 from a regularly scheduled 5.4-m<sup>3</sup> (150 bu) shipment of spawner clams from western Long Island Sound to the waters of Brookhaven Township in Great South Bay. All clams larger than 48 mm shell thickness, were marked with spray paint and planted on the same day at a marked location in eastern Great South Bay (Figure 1). For comparison, weekly sampling of native clams had begun in May from a site about 2 km west of the transplant site (Figure 1). After the transplant, 20 native and 15 spawner clams were sampled simultan-

eously from their respective sites on approximately a weekly basis for the remainder of the summer. A second summer's sampling for both groups was planned; however, no marked spawner clams could be located after the winter, and no new spawner transplants were brought into the Town of Brookhaven in 1979. Therefore, only native clams were sampled during the summer of 1979. During the sampling 228 native females were examined in 1978, 162 transplanted females in 1978, and 195 native females in 1979.

The clams were harvested using commercial clam tongs. They were stored on ice and transported to the laboratory for histological processing. The entire visceral mass was fixed in Bouin's solution for 48 hours. A 1-cm-thick cross section was then excised at the midpoint of and perpendicular to an imaginary line connecting the two adductor muscles (Keck et al. 1975). The tissue sample was prepared for embedding using standard histological techniques (Humason 1967). After transfer through an ascending alcohol series, the samples were embedded in Paraplast® and were sectioned at 10  $\mu$ m. Two slides were prepared from each sample. The slides were stained with Delafield's hematoxylin and counterstained with eosin-Y (Keck et al. 1975).

Five arbitrary stages of female gonadal development were established: (1) "indifferent" (no apparent reproductive

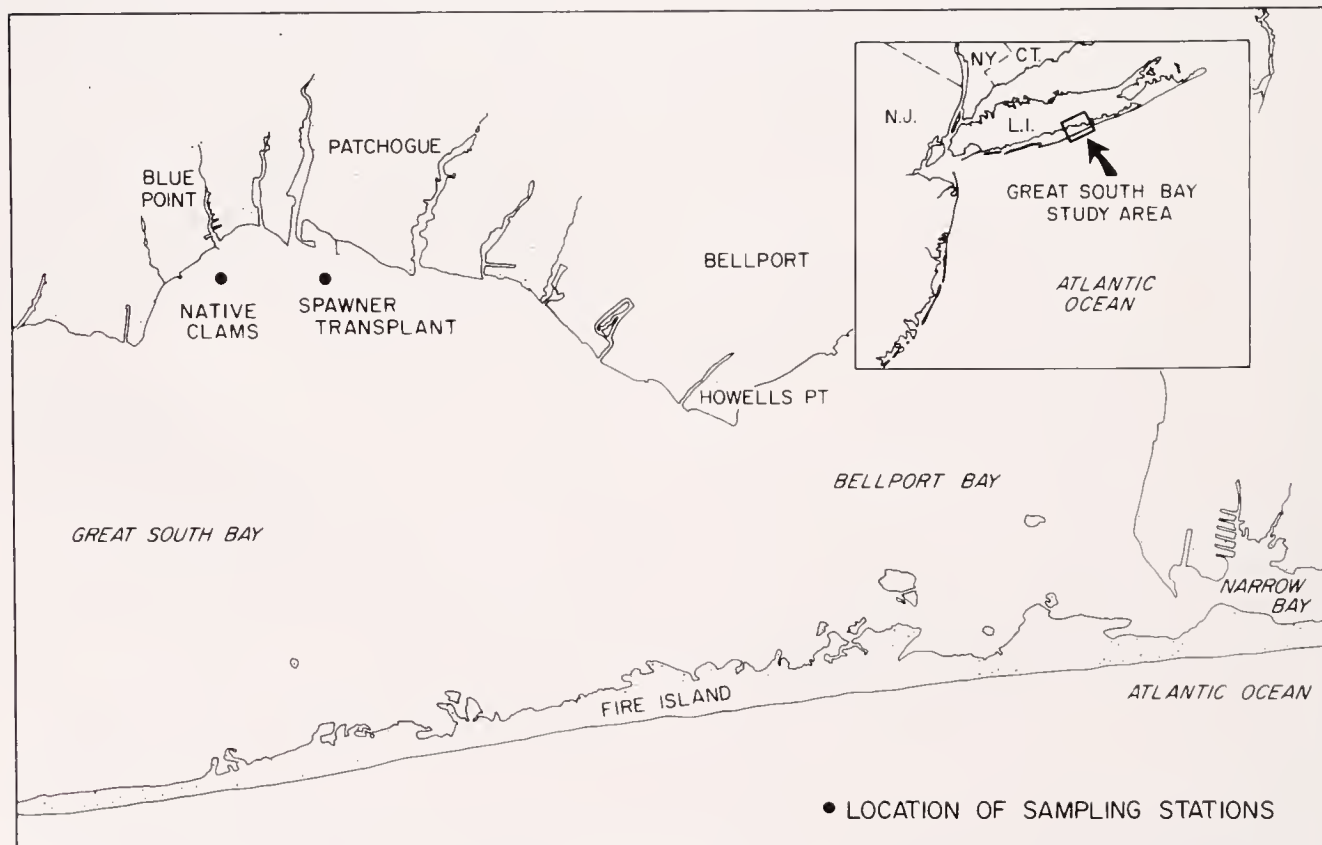


Figure 1. Location of the study site in Great South Bay, Long Island, New York. Shellfisheries in the eastern portion of the Bay, shown here, are managed by the Town of Brookhaven.

activity); (2) "developing" (production of oocytes underway); (3) "mature" (oocyte production ceased, maturation of oocytes progressing); (4) "spawning" (release of gametes commenced); and (5) "spent" (spawning completed). Assignment of gonadal tissue samples to the five categories was based on published work describing morphological changes in the oocytes and ovaries of hard clams at various stages of development (Loosanoff 1937, Porter 1964, Keck et al. 1975). For both the spawner clams and the native clams, the percentage of individuals falling into each of the five developmental classes was calculated for each sampling date. Only the female clams were included in the analysis, primarily because assignment of male gonads to developmental stages on the basis of morphology alone was found to be unreliable.

To facilitate comparisons between native and spawner clams, a gonadal index was calculated based on a method described by Kennedy (1977). Each developmental stage was assigned a numerical value as follows:

Indifferent	0.0
Developing	3.0
Mature	4.0
Spawning	2.0
Spent	1.0

For each sampling date, the number of clams assigned to each stage was multiplied by the appropriate numerical value for that stage. These products were then summed and divided by the total number of clams in the sample. Because the index is essentially an estimate of the spawning potential of the clam, an increasing index precedes spawning, and the index declines rapidly once spawning is initiated.

## RESULTS

Histograms of gonadal condition and calculated gonadal indices for transplanted and native clams during 1978 are given in Figures 2a and 2b, respectively. Note that when sampling of the native clams was begun in May, all of the females were classified as mature, and the gonadal index was at its maximum value of 4.0. This condition persisted through the middle of June. At the time of transplantation (15 June) all of the spawner clams were also classified as mature and had a gonadal index of 4.0.

Spawning of both the native and transplanted clams began sometime between 21 and 26 June. During that interval, the gonadal index began to decline significantly as the number of native females classified as spawning increased from 0 to 22% of those examined. The remainder of the native clams were classified as mature. In that same interval, the fraction of transplanted clams classified as spawning increased from 0 to 33%. In fact, among the transplanted clams sampled on 26 June, 27% were classified as spent. From 21 June to 8 July, the gonadal index of the transplanted clams declined from 4.0 to 1.0, where it remained for the rest of the summer. Spawning by both the native and the transplanted

clams appeared to conclude about 3 July. At that time 87% of the native clams and 100% of the transplanted clams were classified as spent.

Through the middle of August, spent females comprised 100% of both the sampled native and the transplanted clams. Redevelopment was initiated among the native clams, and by mid-September, 36% of the females were classified as developing. No redevelopment was noted among the transplanted clams; they remained classified either as spent or as indifferent.

For comparison with the previous year's pattern, the gametogenic cycle of native clams during the summer of 1979 is given in Figure 2c. In early May, 70% of the native females were classified as developing, and a few were already considered mature. By the end of May, 96% of the females were mature, and the gonadal index had reached 3.8. Spawning was initiated between 29 May and 5 June, about 17 days earlier than the 1978 spawning. During that interval in 1979, the number of spawning females rose from 0 to 15% of those sampled, and the gonadal index declined to 2.3. On 17 June, 81% of the females were classified as spawning, and the rest were considered mature. By 25 June, however, 96% of the females were classified as mature, and the gonadal index increased to 3.8. This anomaly seemed to result from changes in the appearance of the gonads in the 25 June sample. The morphological changes observed in that sample made it difficult to state with any degree of certainty that gamete release had begun. There was no evidence of additional oocyte production, and this should not be considered a second spawning.

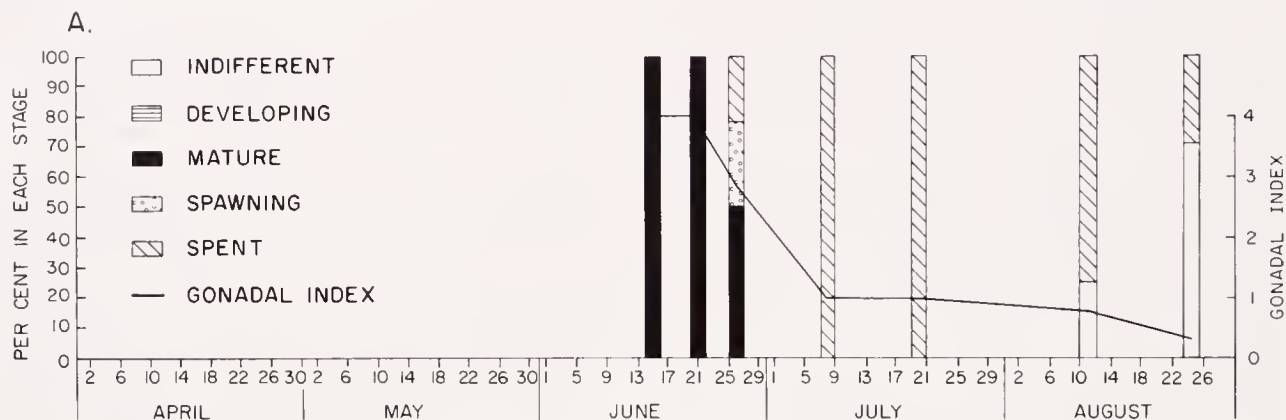
The maximum fraction (71%) of spent females in 1979 was observed in the 10 July sample, by which time the gonadal index dropped to 1.4. The relative abundance of spent females did not increase further. That date was considered the end of the spawning period. As in 1978, redevelopment of the native clams began in August.

Patterns of spawning activity were easily seen in the changing relative frequencies of spawning and spent females as the spawning season progressed. In 1978, the pattern of change of these frequencies was very similar for the transplanted and native clams (Figures 3a and 3b). Among both populations, the frequency of spawning females remained quite low throughout the summer, never exceeding about 25% of any sample. Shortly after spawning females first appeared in the samples (5 June 1978), the frequency of spent females peaked rapidly, reaching a maximum of 100% by August. The 1979 pattern for native clams differed considerably from that found in 1978 (Figure 3c). The frequency of spawning females reached a peak of 75% in the 14 June sample. Conversely, the frequency of spent females in the 1979 samples did not increase as sharply nor reach as high a maximum as it did in 1978.

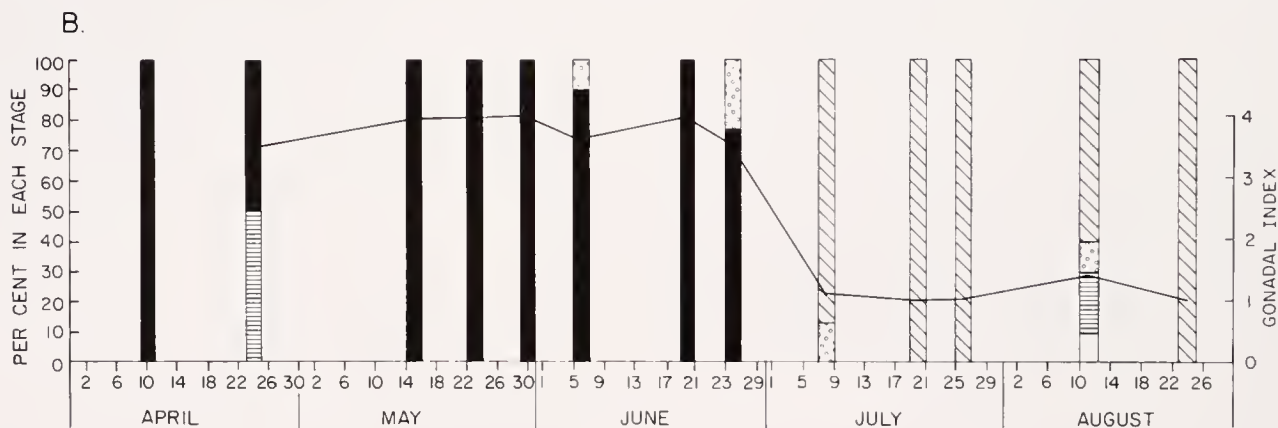
For purposes of this study, the spawning period was defined as the time between the sampling date immediately

## GAMETOGENIC STAGES OF FEMALE HARD CLAMS

SPAWNERS, 1978



NATIVE CLAMS, 1978



NATIVE CLAMS, 1979

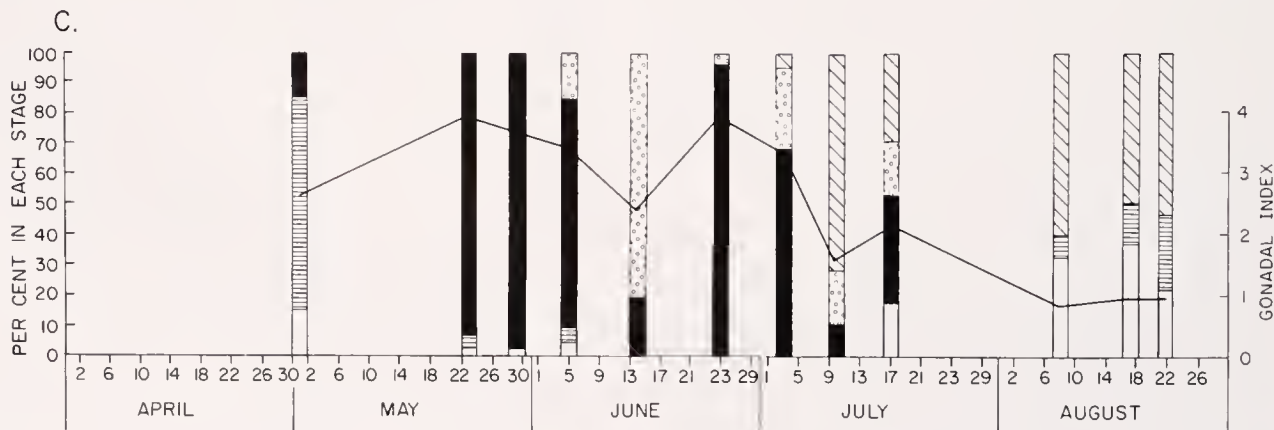


Figure 2. Histograms showing temporal changes in the relative proportions of clams classified as indifferent, developing, mature, spawning, and spent from samples of: (A) spawner transplants, 1978; (B) native clams, 1978; and (C) native clams, 1979. Calculated gonadal indices are also shown.

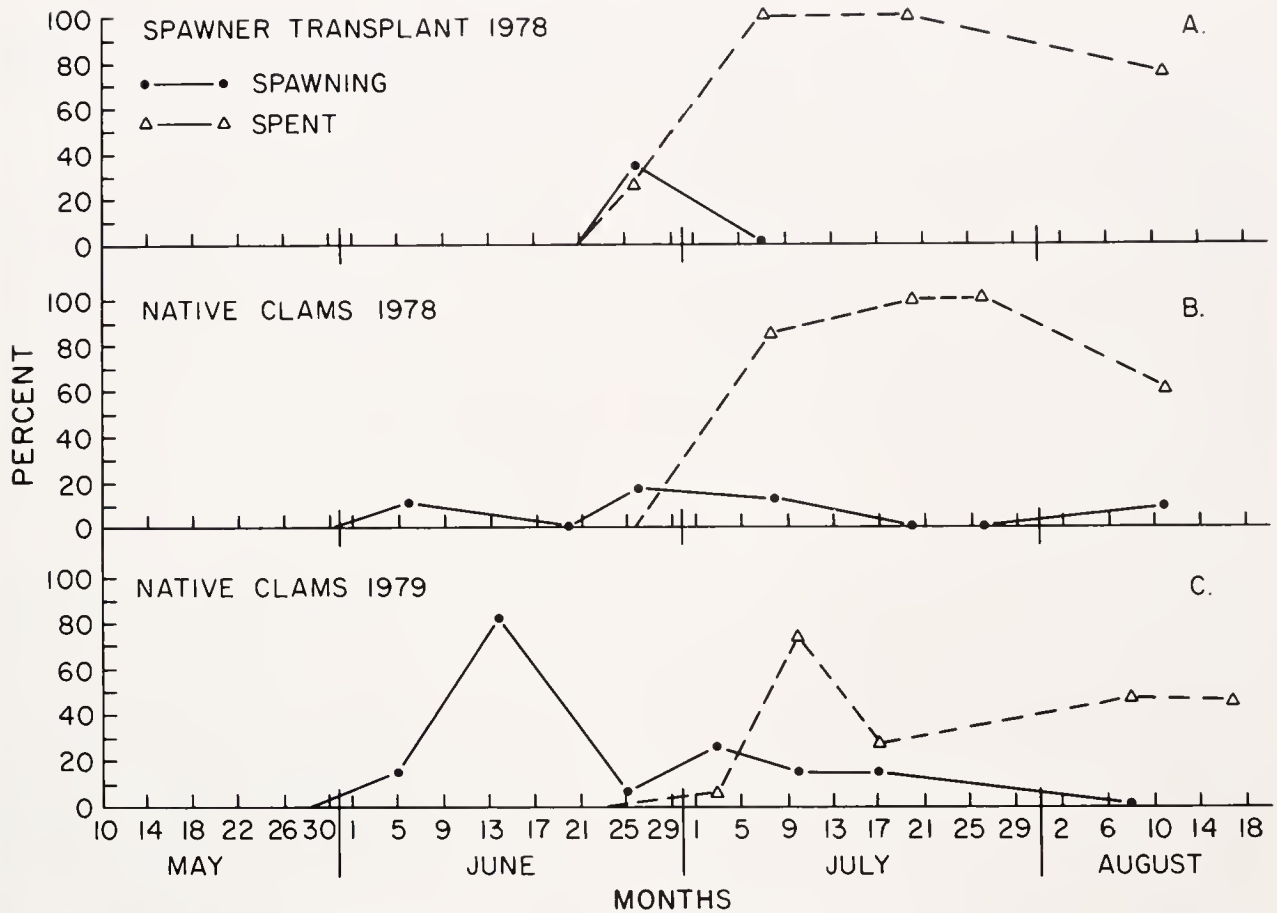


Figure 3. Temporal changes in the percentage of clams classified as spawning and as spent from samples of : (A) spawner transplants, 1978; (B) native clams, 1978; and (C) native clams, 1979.

preceding the first appearance of spawning females and the sampling date when the maximum frequency of spent females was noted. It is clear from our data that the spawning periods for the native and transplanted clams overlapped totally in 1978 (Figure 4). On the other hand,

the spawning period for the native clams in 1979 was quite unlike that of 1978. In 1979, spawning began about 17 days earlier than it had in 1978, although it ended approximately the same time both years.

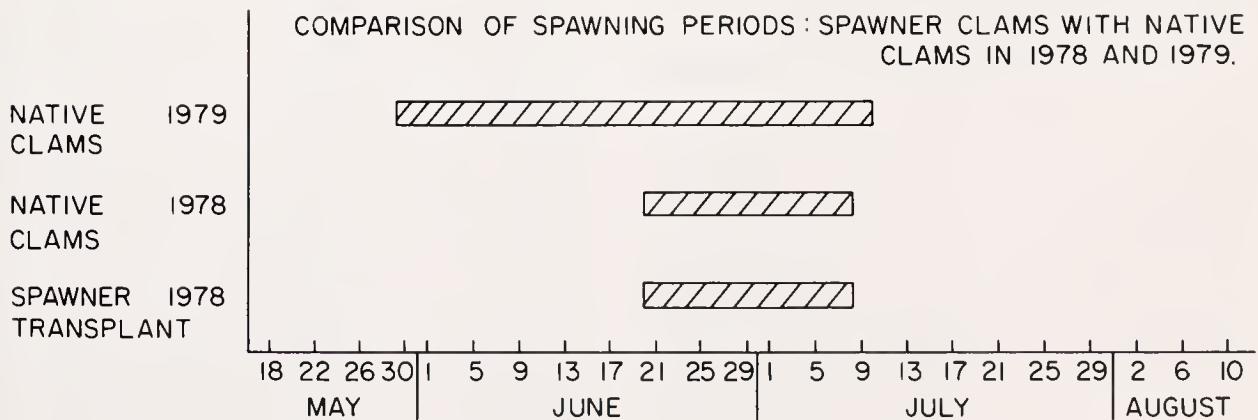


Figure 4. Periods of active spawning by transplanted clams in 1978 and by native clams in 1978 and 1979.

## DISCUSSION

There are many assumptions implicit in the spawner transplant concept, and two of the four basic assumptions tested by this study were invalid during the 1978 Brookhaven transplant. The assumptions that transplanted clams had not spawned prior to being transplanted and that these clams did, in fact, spawn after being transplanted were both valid. On the other hand, the assumption that the native clams have a defined and predictable spawning period was not supported by our data. Further, our data clearly indicated that the native and transplanted clams spawned at the same time in 1978. Consequently, the conceptual basis for the spawner transplants, that they provide larvae for the system at a time when they would not otherwise be present, was not substantiated.

Our data showed that in order to have ensured that transplanted clams spawned only after the native clams had completed their spawning, the 1978 and 1979 Brookhaven transplants should have been carried out after the middle of July. This requirement creates problems for the fishery manager who has only a limited source of spawner clams. Hard clams from states south of Long Island spawn through October (Porter 1964, Eversole et al. 1980), but transplanting from those states into New York waters is not presently permitted by the State of New York (Pieter Van Volkenburgh, NY Dep. Environ. Conserv., personal communication). Therefore, potential sources of spawner clams include only Massachusetts, Rhode Island, and Long Island Sound and its tributaries.

The annual variability of the timing of spawning in Great South Bay, compounded with similar variability elsewhere, makes the optimum time for transplanting very difficult to predict in advance. The spawning season of clams in Massachusetts extends from mid-June to mid-August (Belding 1931). In Rhode Island, spawning by clams occurs in June and July, although in one area it extends into September (Landers 1954). In the tributaries of Long Island Sound, spawning is completed by mid-July, but in the Sound itself spawning apparently does not peak until the latter part of August (Loosanoff 1937). Clearly, if the Brookhaven transplant had been carried out after mid-July, as was apparently necessary to ensure that it occurred after the native clams had spawned, there would have been considerable risk of prior spawning by the potential transplants in their native environments.

Theoretically, the possibility exists of making a contribution to the Great South Bay hard clam resource through spawner transplants carried out before the native clams spawn, rather than after they spawn as is the current practice. The problems are, however, much the same in either case. From our data the onset of spawning is at least as difficult to predict as its completion in Great South Bay. Further, this would require obtaining transplants that are capable of spawning in Great South Bay as early as the first week of

May. Even if clams capable of early May spawning could be legally transplanted to New York from, for example, South Carolina (Eversole et al. 1980), Great South Bay's 12- to 20°C water temperature at that time (Table 1) would probably inhibit spawning until later in the season.

Determination of the causes of the observed annual variation in the timing of gametogenesis and spawning was beyond the scope of this study. It is known that temperature plays a critical role in the timing of gametogenesis in *Mercenaria* (Loosanoff 1937, Porter 1964, Keck et al. 1975, Eversole et al. 1980). It is possible that temperature differences alone might explain the observed annual variation in clam spawning. Ambient temperature data obtained from the nearby Blue Points Co., Inc., showed some differences between the two years, especially in May (Table 1); however, ambient temperatures were virtually identical during the spawning periods in 1978 and 1979.

Studies of gametogenesis in *Mytilus edulis* Linné (Newell et al. 1982) and *Argopecten irradians* (Lamarck) (Sastry 1968, Sastry and Blake 1971) suggested that the interaction between temperature and food availability has a controlling influence on gametogenesis in bivalves. The present study did not include collection of data on food availability at the study site; however, based on measurement of chlorophyll *a*, Lively (1981) reported considerable difference in both the timing and magnitude of the 1979 and 1980 spring algal blooms in Great South Bay. Such variability in the patterns of food availability could contribute to annual variations in

TABLE 1.

Weekly means of the ambient temperature of Great South Bay water pumped into the Blue Points Co., Inc., facility in West Sayville, NY, during the spring and summer of 1978 and 1979.

Week Beginning	1978 Temperature (°C)	1979 Temperature (°C)
Apr 1	8.6	8.6
Apr 8	9.3	8.1
Apr 15	10.2	9.8
Apr 22	12.4	—
May 1	12.2	—
May 8	13.8	18.0
May 15	13.9	17.7
May 22	17.5	18.4
Jun 1	21.2	20.5
Jun 8	20.1	21.8
Jun 15	20.0	21.5
Jun 22	22.5	20.9
Jul 1	22.3	21.9
Jul 8	23.4	23.8
Jul 15	23.7	26.4
Jul 22	26.0	26.6
Aug 1	23.5	28.4
Aug 8	25.4	24.1
Aug 15	26.7	20.1
Aug 22	25.1	23.5

clam gametogenesis and spawning. This would considerably complicate the task of predicting the time of spawning by native clams.

In any case, the numerical contribution that spawner transplants can make to recruitment in Great South Bay is questionable. Because of cost constraints, the typical transplant involves 18 to 36 m<sup>3</sup> (500 to 1000 bu) of large adult clams. Assuming that there are about 7,000 clams/m<sup>3</sup> (250/bu), a large transplant effort would involve about 250,000 clams, of which about 125,000 are probably females. The larger Great South Bay clams annually release roughly  $6 \times 10^6$  eggs (Bricelj and Malouf 1981). Therefore, about  $7.5 \times 10^{11}$  larvae could be produced by such a transplant. McHugh (1981) conservatively estimated that the standing stock of hard clams in the waters of the Town of Islip, which are very similar in area to those of the Town of Brookhaven, produced about  $5.5 \times 10^{15}$  eggs per year. Under those circumstances, a 36-m<sup>3</sup> (1000-bu) transplant in Islip waters could contribute no more than about 0.01% of the larvae produced by the native clams.

Another way of looking at the maximum quantitative contribution from a spawner transplant is through estimates of recruitment. Estimates of recruitment rates of hard clams in Southampton Waters, England, were 8, 5, and 3% of standing stock for three stations (Hibbert 1976). Smith (1979) estimated that annual hard clam recruitment rates in Great South Bay were about 8% of standing stock. If that value is applied to the "standing stock" of a 36-m<sup>3</sup> (1000-bu) transplant, then such a transplant would contribute only about 20,000 harvestable clams, or about 0.02% of the contribution expected from the 915 million native clams (McHugh 1981) in the Town of Islip.

The potential contribution from transplanted spawner clams is difficult to estimate for a number of reasons. There are few estimates of the survival of the larvae of hard clams or of any bivalve. In his classic studies of hard clam populations in Little Egg Harbor, New Jersey, Carriker (1961) reported an average survival of about 2% from early to late planktonic stages; however, his data showed considerable variation and a number of cases where 0% survival was noted. Further, reproduction and successful recruitment are known to be episodic rather than annual in hard clam populations (Greene 1978, Kennish 1978). In studies of *Mya arenaria* Linné, Brousseau (1978) showed that spawning events producing the largest number of larvae did not result in the largest number of recruits. Other studies have demonstrated that among some bivalves there is no consistent relationship between the number of larvae that set at a location and the number that survive to recruitment (Muus 1973). If this was true for hard clam populations in Great South Bay, then the probability of making a significant and

consistent contribution to recruitment through spawner transplant would be further reduced.

Additional uncertainty is introduced when one considers the condition of gametes produced by clams that have been harvested, moved, and released into a new environment. It has been shown that stress on adult bivalves can adversely affect the survival of their offspring (Bayne 1972). In addition, the movement of spawner clams into the warmer waters of Great South Bay could cause them to spawn prematurely. Lannan (1980) clearly demonstrated an optimum temporal "window" in *Crassostrea gigas* (Thunberg) during which larval survival is greatest. Kraeuter et al. (1982) reported that small hard clam eggs have a reduced survival probability. Bricelj and Malouf (1981) showed that the size frequency distributions of Great South Bay hard clam egg size are bimodal and that smaller eggs are released later in the spawning season.

In summary, serious problems with some of the basic assumptions of the spawner transplant concept have been pointed out by this study. In addition, it can be shown that, even under favorable conditions, it is difficult to demonstrate that spawner transplants of the size currently undertaken can make significant contributions to baywide recruitment, unless the larvae produced by such transplants have vastly greater survival probabilities than those produced by the native clams. There are, however, valid reasons to hypothesize that the survival rate of larvae resulting from a transplant might actually be lower than the survival rate of the native larvae.

Although it appears unlikely that spawner transplants can enhance recruitment in an area as large as Great South Bay, it is possible that they could provide larvae to seed specific areas. This practice, the strategic placement of spawning animals, is not new. It is conceptually different from the spawner transplants discussed above in that it does not require that transplanted animals spawn at any particular time after they are correctly positioned on the bay bottom. The practice does, however, involve another set of complex and essentially untested assumptions relating to the behavior, dispersion, and survival of larvae.

#### ACKNOWLEDGMENTS

The authors thank personnel of the Town of Brookhaven for their assistance in the study, Mitzi Eisel for drafting the figures, and Mary Jane Hamilton for typing the manuscript. The authors are also grateful to Jean MaComber, Cornell University, College of Veterinary Medicine, for her help in improving our histological methodology. Support for the study was provided by the Town of Brookhaven and by a grant from the NOAA Office of Sea Grant through the New York Sea Grant Institute.

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## COMMERCIAL POTENTIAL OF CULTURED ATLANTIC SURF CLAMS (*SPISULA SOLIDISSIMA* DILLWYN)

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**ABSTRACT** One-year-old, 60-mm cultured Atlantic surf clams *Spisula solidissima* were investigated as a potential source of clams to meet the steadily growing United States demand for edible clams. Minimal handling or processing was necessary prior to in-shell freezing in relatively low-cost plastic bags. The live, freshly prepared product was organoleptically comparable to hard-shell clams, *Mercenaria mercenaria*, and soft-shell clams, *Mya arenaria*. Frozen storage for 5 months resulted in little flavor deterioration. Compositional and flavor analyses indicate that the small surf clams would be competitive in the marketplace.

### INTRODUCTION

The initial results of a previous study (Krzynowek et al. 1980) indicated that young, cultured surf clams, *Spisula solidissima* (referred to as yearlings), were an acceptable product to taste panelists, comparable to and, therefore, probably competitive with more familiar clam species (e.g., hard clams), and one that could be successfully frozen. The yearling clams, frozen raw in the shell, appeared commercially attractive. Those preliminary results indicated that the product was easy to shuck, and the raw meat yield was 25%.

The present study was designed to corroborate the preliminary report by using a different year class of cultured yearling surf clams. The clams were tested organoleptically and chemically as a fresh product and at varying times during frozen storage. They were frozen whole in the shell.

### MATERIALS AND METHODS

Surf clams grown to a size of 60 mm in a flow-through system at the National Marine Fisheries Service (NMFS) Milford Laboratory, Milford, CT, were transported live to the Northeastern University Marine Research Laboratory, Nahant, MA, for depuration and maintenance. Determinations were made for organoleptic acceptance, chemical composition, and frozen storage stability.

To determine if yearling surf clams could compete in the marketplace with soft- and hard-shell (northern quahog) clams, members of the Gloucester Laboratory taste panel were asked if they could distinguish differences between steamed surf and soft-shell clams, between fried surf and soft-shell clams, and between raw, half-shell surf and hard-shell clams ("cherrystones" and/or "littlenecks"). Differences were rated as *great*, *moderate*, *slight*, *very slight*, or *no difference*. Preferences for the yearling surf clams were rated as *more acceptable*, *less acceptable*, or *comparable to other clams*. Difference/preference tests were conducted with fresh surf clams at time zero.

Chemical composition was determined on a homogeneously blended composite of 24 raw, shucked, and drained

yearlings. All chemical tests were performed as described previously (Krzynowek et al. 1980).

To determine the storage stability of surf clams frozen in-shell, 24 whole, live clams were vacuum packed in plastic bags and frozen at  $-30^{\circ}\text{C}$  for 24 hours. The bags were then stored at  $-20^{\circ}\text{C}$ . At predetermined intervals, clams were removed from frozen storage, thawed, and analyzed chemically and organoleptically.

Chemical changes which occurred during storage were assessed by comparing compositions of fresh, live yearlings to frozen yearlings over a 12-month storage period. Samples were prepared for analyses by removing one package of 24 yearlings from frozen storage, thawing, shucking, draining, and blending the clams until homogeneous. Aliquots were taken from the homogeneous mixture. Chemical methods for proximate analyses were described previously (Krzynowek et al. 1980). Sample variation over the months of storage was tested by analysis of variance as being significant at  $P < 0.01$ , and points of difference were detected by Duncan's (1955) multiple range test.

Organoleptic changes were assessed using a 12-member taste panel of Gloucester Laboratory personnel. The yearlings were served steamed, and their appearance (A), odor (O), flavor (F), and texture (T) were graded on a 9-point scale (9—excellent; 5—borderline; 1—inedible). Numerical ratings were used as the only indication of product quality because, unfortunately, the fresh controls held live in Nahant, MA, died from starvation. Significant differences among scores through the 5 months of organoleptic testing were tested by analysis of variance at  $P < 0.01$ .

### RESULTS AND DISCUSSION

Moderate differences were noted between the steamed surf and soft-shell clams (Table 1). Slight differences were detected between the fried clam products and between the raw clams served on the half-shell. There was a slight difference for soft-shell clams as a steamed product and a 50/50 rating (comparable/preferred soft-shell) between the two fried products. Differences and preferences were both

TABLE 1.

Results of taste panel comparison of fried and steamed surf and soft-shell clams, and of raw surf and hard-shell clams on the half-shell.

Responses	Number of Panelists		
	Product Form		
	Fried	Steamed	Raw
Differences:			
great	0	0	2
moderate	3	8	2
slight	5	3	4
very slight	3	1	1
no difference	1	0	1
Preferences (surf clams):			
more acceptable	0	1	2
comparable products	6	3	4
less acceptable	6	8	4

related to the texture of the yearling surf clams. When the yearlings were not the preferred species as steamed or fried clams, it was because they were considered tough or chewy in texture. In appearance, odor, and flavor surf clams were comparable to other steamed or fried clams. Surf clams

were generally comparable to hard-shell clams as a raw product served on the half shell. Panelists who preferred yearlings felt they were easier to chew than cherrystones or littlenecks (hard-shell clams).

The fresh raw product contained 82.5% moisture, 2.4% ash, 0.7% fat, and 12.8% protein (Figure 1). Carbohydrate (1.6%) was calculated by difference. Calories were calculated to be 64 kcal/100 g of raw surf clam. These values were comparable to values found for soft- and hard-shell clams (Sidwell 1981). Meat yield for the raw, shucked product was about 15%.

Changes in moisture, total protein, and extractable protein during storage are shown in Figure 1. As in the previous study, there was a significant uptake of moisture by the flesh of the surf clams from  $82.51 \pm 0.04\%$  during the first month of storage to a mean, constant value of  $88.03 \pm 0.90\%$  for the remainder of the storage time. In fact, shucked yields rose to 23 and 24% after one and two months of storage, respectively. This water uptake probably occurred within the first nine days of storage according to other postmortem changes in muscle properties as reported by Lee et al. (1978). Total protein also appeared to decrease sharply in the first month, from  $12.80 \pm 0.78\%$  to a mean, constant value of  $8.31 \pm 1.03\%$  for the remainder

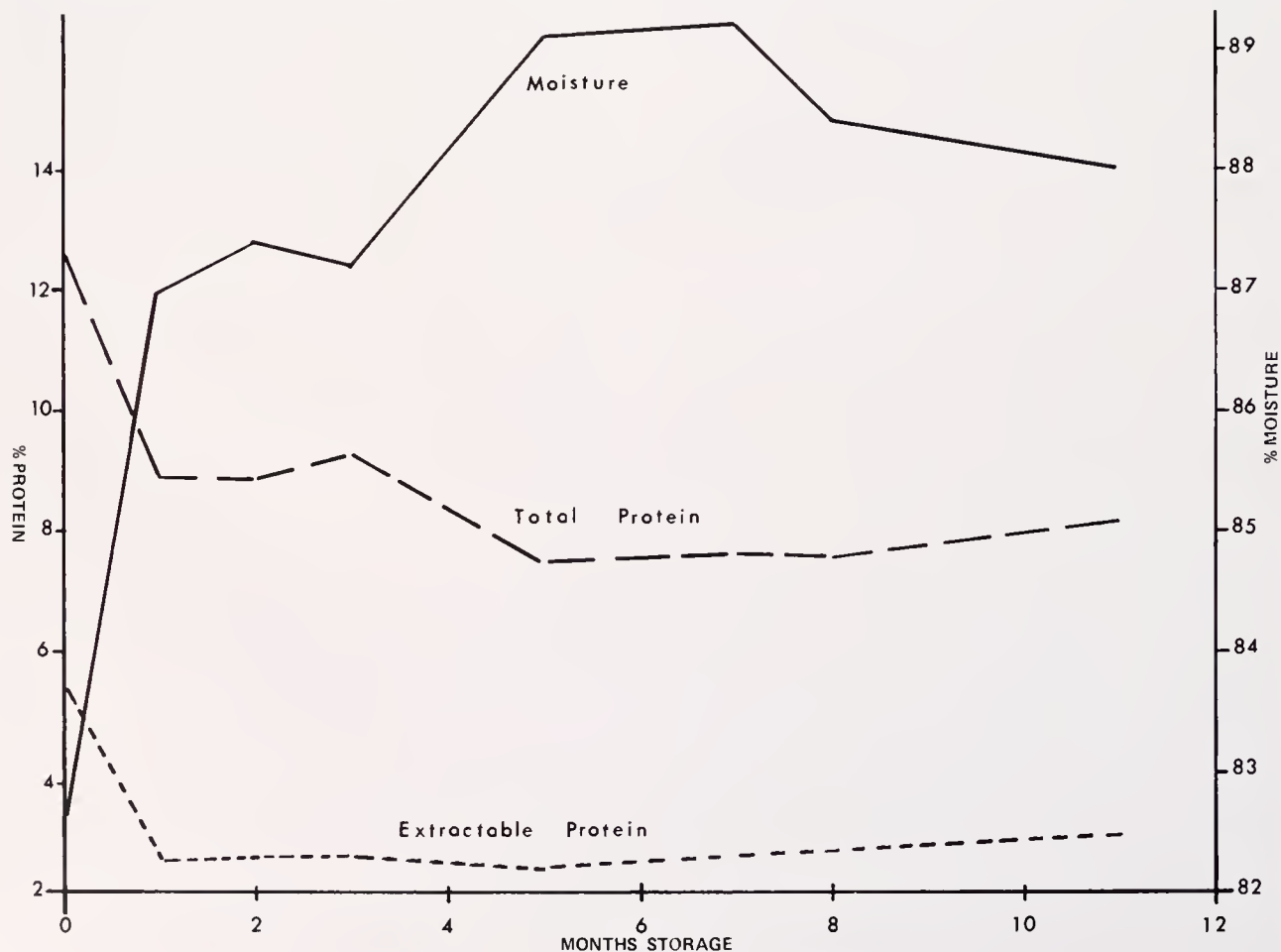


Figure 1. Changes in moisture and total and extractable proteins during frozen storage.

of the storage. The large moisture increase accounted for the protein decrease. On a dry-weight basis, total protein remained the same. Therefore, no protein was lost to the liquor. The decrease in extractable protein, however, cannot be totally explained by moisture gain. After the initial decline from  $5.64 \pm 0.03\%$  to  $2.55 \pm 0.07\%$ , the amount of extractable protein remained the same throughout the year of frozen storage at a mean value of about 2.6%. The mean fat content ( $0.67 \pm 0.21\%$ ) and the mean ash content ( $2.68 \pm 0.28\%$ ) remained constant during storage.

The overall (mean) taste test scores of A, O, F, and T never fell below 6.2 (fair) through the five months of organoleptic testing (Table 2). The storage scores were not significantly different from the fresh controls served simultaneously with the stored samples. The individual scores for some months were widely divergent among the 12 panelists, primarily because the surf clams were an unfamiliar species. Comments on the score sheets indicated that some scores reflected a comparison to familiar clam species even though the more familiar clams were not included in the taste test.

TABLE 2.  
Organoleptic scores for steamed surf clams.

Months in Frozen Storage	Appearance	Odor	Flavor	Texture	Mean AOFT
0	$7.7 \pm 1.2$	$7.8 \pm 1.1$	$7.1 \pm 1.4$	$6.8 \pm 1.7$	$7.6 \pm 1.2$
1	$7.7 \pm 1.6$	$7.3 \pm 1.1$	$6.4 \pm 1.4$	$7.1 \pm 1.0$	$7.1 \pm 1.3$
2	$7.9 \pm 0.5$	$7.3 \pm 1.0$	$6.7 \pm 1.2$	$6.4 \pm 1.4$	$7.1 \pm 1.2$
3	$7.1 \pm 0.9$	$6.9 \pm 1.3$	$5.6 \pm 1.4$	$5.8 \pm 1.5$	$6.3 \pm 1.4$
5	$7.0 \pm 1.6$	$6.9 \pm 0.9$	$5.7 \pm 1.6$	$5.7 \pm 2.0$	$6.2 \pm 1.8$

The small surf clams were acceptable as fried, steamed, and especially raw, half-shell products. The most unacceptable feature was the tougher texture encountered when they were steamed and/or fried. This problem was subsequently solved by reduced cooking times. They were comparable and, therefore, could compete in the marketplace with the more familiar soft- and hard-shell clams.

The surf clams were held frozen for at least five months with little loss in their organoleptic quality. They were frozen with a minimum of prehandling and processing. Plastic storage bags cost less than the rigid containers normally used for packaging of shucked products with broth. Frozen clams were shucked with ease, and should be adaptable to mechanical shucking. The thawed and drained, frozen-shucked product even had a substantial weight gain over its fresh, live counterpart because of a high uptake of shell liquor into their bodies during the first month of storage. After the first month's weight gain and extractable protein loss, their proximate composition remained constant for the remainder of the year during storage.

Organoleptic and chemical analyses indicate that yearling surf clams have excellent market potential as both live and frozen products. They are low in fat, tasty, and very versatile. Market testing and economic studies are scheduled to determine the feasibility of marketing this clam species.

#### ACKNOWLEDGMENTS

The authors express their appreciation to Gloucester Laboratory personnel who participated in the taste-test panel, and to the personnel at Northeastern University Research Laboratory in Nahant, MA, who maintained and depurated the clams.

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## INFLUENCE OF REDUCED SALINITY ON THE ATLANTIC BAY SCALLOP *ARGOPECTEN IRRADIANS* (LAMARCK) AT VARIOUS TEMPERATURES

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**ABSTRACT** The short-term effects of reduced salinities on bay scallops were investigated by exposing animals to salinities of 0, 5, 10, and 15 ppt for periods of 2, 6, 24, and 48 hours. The experiment was repeated during various times of the year at ambient seawater temperatures of 24°, 19°, 13°, 5°, 1°, and 0°C. Survival generally decreased for a given exposure time as the temperature increased. At 24°C total mortality occurred at salinities of 0 to 10 ppt after 6 hours. None of the animals at 19° and 13°C survived after 24 hours in 0 to 5 ppt. Bay scallops at 5°C exhibited total mortality after 24 hours of exposure at 0 ppt. The mean mortality was 30% or less after exposure to all time and salinity combinations at 1°C. Results at 0°C were inconclusive. In general, scallop survival in 15 ppt was 80% or better at each time and temperature. Mortality was greatest in combinations of low salinity (0, 5, 10 ppt) and high temperature (24°, 19°C). Response surface curves based on data were used to make predictions concerning scallop survival at salinities from 0 to 20 ppt and exposures up to 50 hours. These observations may explain mortalities in natural scallop populations during heavy freshets.

### INTRODUCTION

Salinity and temperature are important environmental factors which influence the distribution and survival of many marine organisms (Vernberg et al. 1963). The bay scallop *Argopecten irradians* (Lamarck) has been observed in salinities ranging from as low as 10 ppt to as high as 38 ppt (Belding 1910, Gutsell 1931, Sastry 1961, Castagna and Chanley 1973, Duggan 1975). These bivalves are commonly found in high-salinity estuaries, bays, and inlets along the eastern and Gulf coasts of the United States (Belding 1910, Gutsell 1931, Duggan 1975). Heavy rainfall and freshwater runoff often expose scallops to greatly reduced salinities. Broom (1976) noted that bay scallops may suffer considerable exposure to freshets and low temperature because they are often found in shallow water areas. Gutsell (1931) maintained that heavy freshets can be very destructive and that severe cold weather, especially when accompanied by spring low tides, sometimes damaged scallop populations. Significant mortality among scallop populations has been observed during the spring and may be a result of these freshets.

Several observations on scallops and salinity have been reported. Sastry (1961) exposed scallops directly to lowered salinities, including 21, 14, 7, and 0 ppt for a 2-hour period. The animals remained active at 21 and 14 ppt and displayed no change in behavior. Although the scallops at 7 ppt initially closed their valves, they later opened them without extending their tentacles. No active water circulation could be observed. After 2 hours of exposure, all animals were returned to ambient (28 ppt) seawater where they resumed normal activity. Scallops submerged in distilled (0 ppt) water also survived but kept their valves tightly closed even after returning to ambient seawater.

Duggan (1975) examined the effects of gradual salinity reductions on bay scallops at temperatures between 10° and 15°C and 20° and 25°C. Animals at ambient (25 to 27 ppt) seawater were acclimated to 5 to 7 ppt over a 4-hour period by adding 5 l of fresh tap or distilled water at 30-minute intervals. During submersion at 15 ppt, the scallops retracted their tentacles and partially closed their shells. No activity was observed during reductions below 15 ppt. The scallops resumed normal activity after being transferred into ambient seawater.

These experiments were designed to determine the relationship between low-salinity exposure, time, and temperature for bay scallops. This information is needed to explain observations on scallop distributions and mortalities in the natural environment and to permit better decisions on planting sites for bay scallops.

### MATERIALS AND METHODS

All experiments were performed with hatchery-reared bay scallops between 10 and 30 mm in height. Fresh water for salinity dilutions was collected from a waterfall on the Wepawaug River, Milford, CT. Seawater was obtained from Milford Harbor and had a salinity between 25 and 28 ppt. Salinity determinations were made using hydrometers and Knudsen's tables. Salinities and temperatures were accurate to  $\pm 1$  ppt and  $\pm 1^\circ\text{C}$ . Tests were made at ambient seawater temperature with scallops that had been held in ambient seawater for at least 1 month. Experiments were performed in standing seawater in 10-l polyethylene pans, each initially containing up to four groups of 10 scallops held in 88- X 88- X 50-mm plastic berry baskets with mesh liners. During a 3-hour period the salinities in the pans were reduced gradually by dilution to 15, 10, 5, or 0 ppt. The

scallops remained at these salinities for 2, 6, 24, and 48 hours, respectively, before being reacclimated to ambient salinity at 5 ppt intervals over a 3-hour period. When ambient salinity was reached, the baskets were placed in flowing seawater for a 1- to 2-week observation period. Control groups at ambient salinity in standing seawater pans were removed at 2 and 48 hours and similarly placed in flowing seawater. The scallops were examined daily, and the dead bivalves removed. Animals were considered dead if they did not respond to tactile stimuli or display movement. Dead scallops were often found gaping widely with loose meats and a fetid odor. Darkening of the blue eyes usually took place and an absence of byssal threads and fecal material could be noted. The experiment was performed once at 19°, 13°, 5°, and 1°C and in duplicate at 24° and 0°C.

Survival data from these tests were used to construct response surface curves for each temperature (Figures 1 through 6). Data points for these curves were determined in the following manner: regression coefficients were estimated by a stepwise regression analysis (using a BMPOZR Program of the Biomedical Computer Programs at the University of California). The regression coefficients were fitted to a full quadratic equation with two variables, time and salinity, using a quadratic equation to give the points plotted in the response surface curves:

$$Y = K + b_1 X_1 + b_2 X_2 + b_3 X_1^2 + b_4 X_2^2 + b_5 X_1 X_2$$

where  $Y = \arcsin \sqrt{\% \text{ survival}}$ ,  $K = \text{a constant}$ ,  $X_1 = \text{time}$ , and  $X_2 = \text{salinity}$ . The number of animals which survived at each salinity, temperature, and time combination is reflected in these curves.

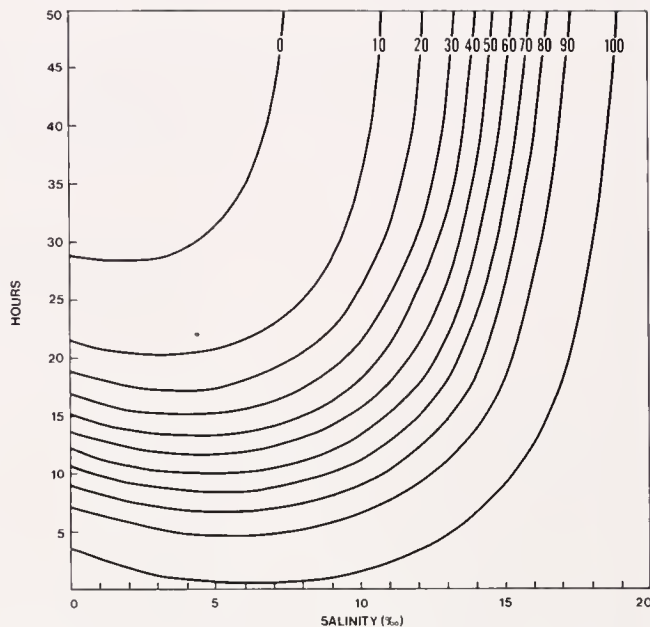


Figure 2. Response surface curve prediction of scallop survival at salinities between 0 and 20 ppt up to 50 hours at 19°C.

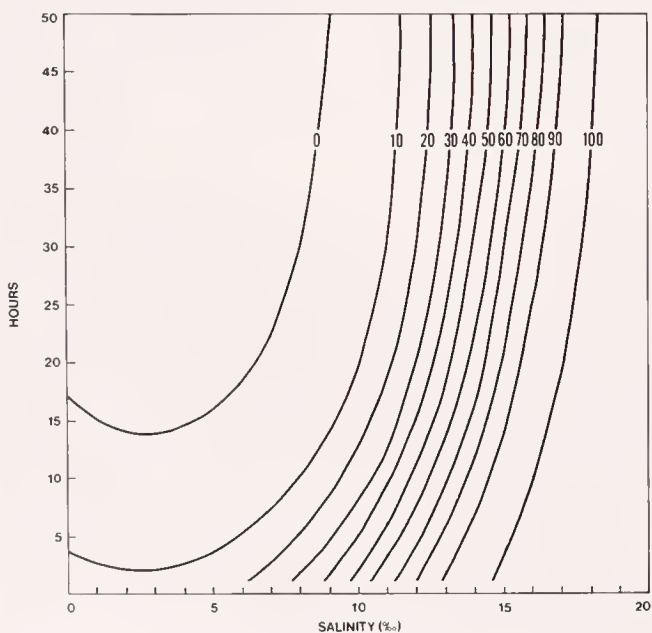


Figure 1. Response surface curve prediction of scallop survival at salinities between 0 and 20 ppt up to 50 hours at 24°C.

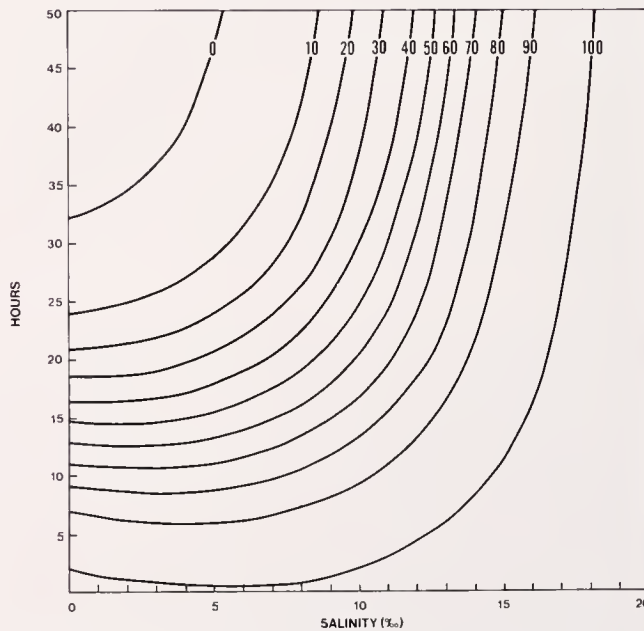


Figure 3. Response surface curve prediction of scallop survival at salinities between 0 and 20 ppt up to 50 hours at 13°C.

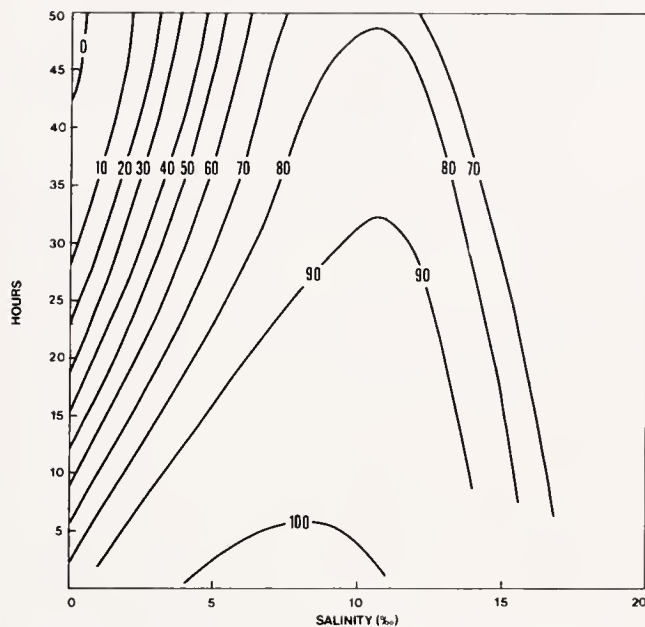


Figure 4. Response surface curve prediction of scallop survival at salinities between 0 and 20 ppt up to 50 hours at 5°C.

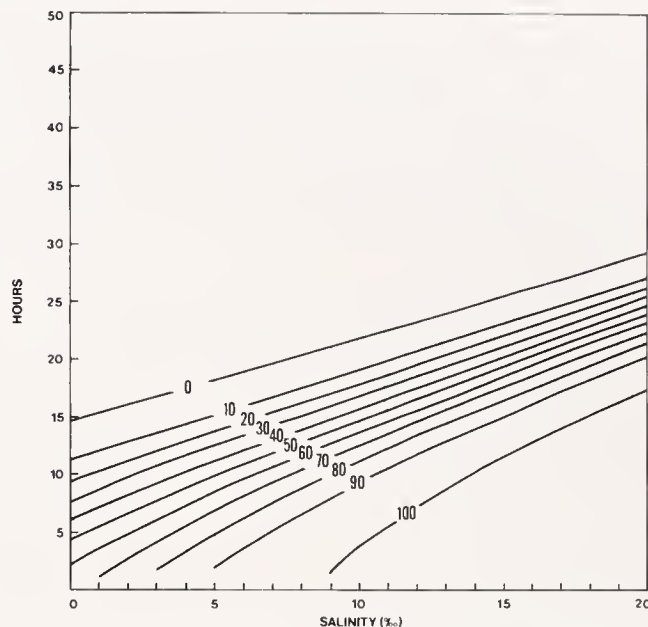


Figure 6. Response surface curve prediction of scallop survival at salinities between 0 and 20 ppt up to 50 hours at 0°C.

## RESULTS

Scallops in duplicate experiments at 24°C experienced total mortality at 0 and 5 ppt when exposed for 5 hours or more, with  $\leq 20\%$  survival occurring at 2 hours. Fewer than 20% of the animals survived exposure for 6 hours or more at 10 ppt. No scallops were able to tolerate submersion at 10 ppt for 24 hours or more. Sixty percent survival or better occurred at 15 ppt for all time intervals (Table 1).

TABLE 1.

Survival of scallops exposed to salinities of 0, 5, 10, 15, and 28 ppt for time intervals of 2, 6, 24, and 48 hours at 24°C and 19°C.

Salinity (ppt)	24°C				24°C				19°C			
	Hours				Hours				Hours			
	2	6	24	48	2	6	24	48	2	6	24	48
28	10	—	—	10	10	—	—	10	10	—	—	10
15	10	9	10	6	9	10	9	9	10	10	10	10
10	10	0	0	0	8	2	0	0	10	10	1	0
5	6	0	0	0	4	0	0	0	10	10	0	0
0	2	0	0	0	1	0	0	0	10	9	0	0

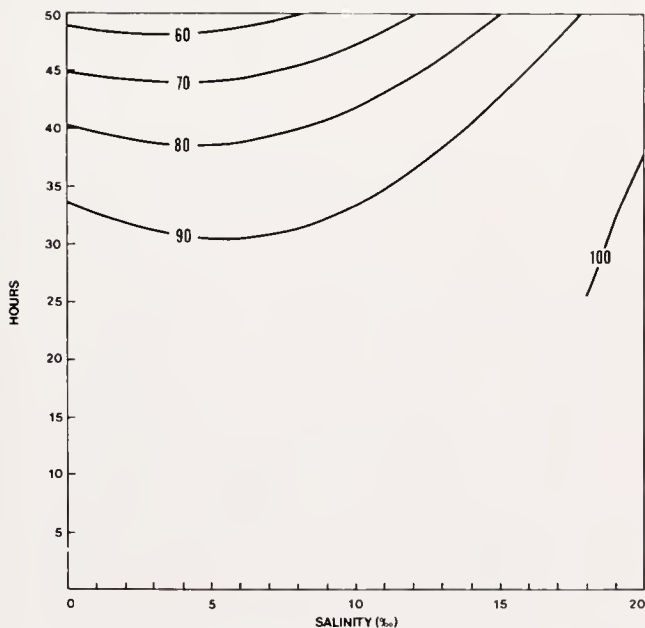


Figure 5. Response surface curve prediction of scallop survival at salinities between 0 and 20 ppt up to 50 hours at 1°C.

Ten percent mortality was found at 19°C in 0, 5, and 10 ppt up to 6 hours. Only one scallop survived submersion at 10 ppt for 24 hours, with no scallops enduring exposure for 48 hours. Total mortality occurred at 0 and 5 ppt for 24 hours or more. All of the animals tolerated 15 ppt for each of the time periods (Table 1).

Better than 90% of the bivalves survived in 0 and 5 ppt at 13°C up to 6 hours. No survival occurred at 24 hours or

more. Mortality was 10% at 10 ppt up to 24 hours. Total survival occurred at 15 ppt for all the time intervals (Table 2).

At 5°C, 90% of the scallops tolerated submersion at 0 ppt up to 6 hours, while complete mortality occurred at  $\geq 24$  hours at this salinity. Some survival occurred at 5 ppt for all of the time intervals with as many as 50% alive at 48 hours (Table 2).

TABLE 2.

Survival of scallops exposed to salinities of 0, 5, 10, 15, and 28 ppt for time intervals of 2, 6, 24, and 48 hours at 13°, 5°, and 1°C.

Salinity (ppt)	13°C				5°C				1°C			
	Hours				Hours				Hours			
	2	6	24	48	2	6	24	48	2	6	24	48
28	10	—	—	9	10	—	—	8	10	—	—	10
15	10	10	10	10	8	9	8	8	10	10	8	9
10	10	10	9	0	10	10	10	8	9	9	10	7
5	10	10	0	0	10	8	9	5	9	10	10	4
0	10	9	0	0	9	9	0	0	10	10	9	7

Some of the bivalves at 1°C survived at all the temperature-salinity combinations. At 0 and 10 ppt, 70% of the scallops tolerated exposure up to 48 hours. Survival at 5 ppt for 48 hours was 40%. At the other time intervals and salinities, 20% mortality or less was observed (Table 2).

During duplicate experiments at 0°C all of the animals at  $\geq 24$  hours in 0 ppt died. Some survival occurred at  $\leq 6$  hours in both groups with as few as 60% alive at 2 hours and 50% alive at 6 hours. Most scallops tolerated exposure to 5, 10, and 15 ppt at all time intervals; however, only 40% survival was found at 5 ppt for 48 hours. Unusual mortality occurred in the control groups at 0°C, with only 60% alive at 2 hours in one of the groups (Table 3).

TABLE 3.

Survival of scallops exposed to salinities of 0, 5, 10, 15, and 28 ppt for time intervals of 2, 6, 24, and 48 hours at 0°C.

Salinity (ppt)	0°C				0°C			
	Hours				Hours			
	2	6	24	48	2	6	24	48
28	9	—	—	10	6	—	—	10
15	10	10	9	8	9	10	7	10
10	7	9	9	7	8	6	7	8
5	8	8	6	5	8	8	9	4
0	6	5	0	0	7	9	0	0

Response surface curves for each temperature are presented in Figures 1 through 6. These curves are explained

in the discussion. Raw survival data are listed in Tables 1 through 3.

## DISCUSSION

Reduced salinity caused by heavy rainfall and ensuing runoff commonly occurs in estuaries inhabited by the bay scallop. These bivalves are able to tolerate exposure to low salinity for varying lengths of time, depending on the temperature.

Brief submersion in fresh water, for as little as 2 hours, is lethal to scallops at warmer temperatures (24°C). Animals at temperatures between 13° and 5°C can withstand exposure to 0 ppt for periods up to 6 hours. Scallops in cold water at 1°C can endure fresh water up to 48 hours. During experimentation, scallops at 0°C did not survive exposure to fresh water for periods  $> 6$  hours. Winter mortality of laboratory-held scallops is not uncommon, and those used in this test may have been stressed from frequent handling and exposure to air temperature. It is possible that the combination of extreme temperature and low salinity created an intolerable environment for the bay scallop. Gunter (1961) stated that salinity is a limiting factor in the distribution of many marine organisms, especially at the lower extremes. However, excellent survival in these experiments at 1°C in fresh water suggests that scallops may tolerate exposure at 0°C in their natural environment during winter and early spring freshets and that the low survivals at 0°C and low salinities in these experiments are attributable to other factors. Significant mortality in the control supports this. Thus, in general, the bay scallops survived submersion in low salinity better at cooler temperatures. Vernberg et al. (1963) also found that specimens of cold-acclimated *Argopecten irradians* and *Modiolus modiolus* (Linné) were more resistant to low salinity than warm-acclimated individuals.

Other bivalves are also more resistant to low salinity at reduced temperature. Castagna and Chanley (1973) noted better tolerance to a range of salinities at cooler temperatures with the false angel wing *Petricola pholadiformis* (Lamarck). Loosanoff (1965) observed that the American oyster *Crassostrea virginica* (Gmelin) can survive periods of spring floods or heavy rains when the salinity of the water is greatly reduced. He noted that temperature is extremely important to this survival because the lower the temperature, the longer the oysters can tolerate low-salinity water. Galtsoff (1964) found that oysters conditioned slowly at low temperature and low salinity can endure prolonged situations of stress. Loosanoff (1948) observed that at temperatures between 8° and 12°C some adult and young oysters survived 70 days in fresh water, while all animals at temperatures between 22° and 26°C died within 13 days. Chanley (1958) studied the effects of reduced salinity on juvenile bivalves. He noted that several factors are of primary importance in determining how long animals can exist in salinities that are too low for indefinite survival.

These conditions include cooler water temperatures, larger animal sizes, and the ability of the bivalve to withdraw into a watertight shell.

In the present study, bay scallops exposed to salinities of 0, 5, and 10 ppt in warm seawater, initially closed their valves tightly. After several hours of submersion at salinities of  $\leq 10$  ppt, the scallops opened their shells without extending their tentacles. As Duggan (1975) reported, scallops exposed to salinities of 15 to 16 ppt fully retracted their tentacles, closed their shells, and ceased all movement. He found that a few scallops did not close their shells completely but closed their mantle edges in what appeared to be an attempt to seal the body tissues from the changing environment.

Bay scallops can endure exposure to 15 ppt for at least 48 hours. Scallops in the control group and at 15 ppt for several hours displayed normal activity, including clapping, full extension of tentacles, and formation of byssal threads. None of the bivalves at  $\leq 10$  ppt acted in a normal manner until they were returned to flowing ambient seawater.

Vernberg et al. (1963) tested the effects of temperature and salinity on excised gill tissue. Gill cilia of *Argopecten* were least resistant to lower salinity among those bivalves tested. They noted a reduction in gill ciliary activity at 18 ppt and a complete cessation of activity below 12 ppt. The response of the gill tissue corresponded with that of the whole animal.

Response surface curves, based on survival data and prepared for each temperature, suggest the probable results of exposure at salinities between 0 and 20 ppt for 50 hours. According to the curves, at 25°C 50% mortality can be expected between 10 and 15 ppt at exposure times as short as 2 hours (Figure 1). In 19°C such mortality should not occur until 16 hours at 10 ppt and 12 hours at 0 to 5 ppt (Figure 2). This trend continues at 13°C where exposure

for 27 hours at 10 ppt and 15 hours at 0 to 5 ppt is necessary to cause a 50% mortality (Figure 3). At 5°C bay scallops are quite resistant to reduced salinity with 80% survival at 10 ppt for 48 hours and 50% survival expected at 42 hours in 5 ppt (Figure 4). However, survival in fresh water at 5°C is no longer than that occurring at 13° and 19°C. Salinity tolerance is greatest at 1°C, where 90% survival is predicted in exposures up to 30 hours and better than 50% survival in exposures up to 50 hours (Figure 5). The 0°C response surface curve reflects a reversal in the trend of increased tolerance to lower salinities as the temperature declines which is apparent from the experiments at 1°C and above (Figure 6). Because this curve and the raw data in Table 3 were probably influenced by other laboratory stress conditions that occurred during the 0°C tests, they may not accurately reflect the consequences of salinity exposures at this temperature.

The salinity-temperature-time experiments provide predictive information for determining whether heavy freshets will produce mortality in scallop populations. This information can explain the absence of scallops in estuaries fed by large rivers with heavy freshwater runoff. When planting bay scallops that are produced by aquacultural methods, it is important to select an estuarine area which is not subjected to frequent and extended exposure to lower salinity.

#### ACKNOWLEDGMENTS

The authors express their appreciation to John MacInnes for running the computer analysis of the survival data and producing the response surface curve points. We also thank Patricia Boyd, Patricia Goertz, Ronald Goldberg, and James Widman for technical help and Rita Riccio for editorial assistance.

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## AMPHIPODS AS A POTENTIAL DIET FOR JUVENILES OF THE AMERICAN LOBSTER *HOMARUS AMERICANUS* (MILNE EDWARDS)

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**ABSTRACT** The amphipod *Gammarus oceanicus* (Segerstråle) was considered as an alternative diet to frozen brine shrimp (*Artemia salina* L.) and compounded diets for juvenile lobsters (*Homarus americanus*). There were no significant differences in the growth rates or mean weight gains between lobsters fed these three diets. Mortality was significantly higher in lobsters fed the compound diet tested in this study. Lobsters that were fed live amphipods had a highly pigmented exoskeleton, giving a deep red-brown coloration. Those that were fed compound diets lacked this pigmentation and were greyish in color. Lobsters that were fed *Artemia* were intermediate in color and pigmentation.

### INTRODUCTION

Although compound diets continue to improve in terms of growth and survival, the brine shrimp *Artemia salina* still remains the best diet for rearing juvenile lobsters. Brine shrimp are becoming increasingly expensive and are variable in quality; however, neither brine shrimp nor the compound diets produce the intense pigmentation of a wild-caught lobster (D'Agostino 1980). Lobsters that consume the compound diet tend to be blue to greyish in color. This is caused by a lack of pigmentation in the exoskeleton. Douglas Conklin (Bodega Marine Laboratory, Bodega Bay, CA; pers. comm.) improved the coloration of lobsters which consumed a compounded diet that incorporated paprika oil into the mix. Hughes and Matthiessen (1962) found that by supplementing a shellfish diet with freshly killed crab, a natural wild-type coloration was obtained in lobsters which were previously blue.

Live amphipods were suggested as an alternative to brine shrimp or compounded diets for feeding postlarval lobsters. For the present trial, the amphipod *Gammarus oceanicus* (Segerstråle) was chosen because it is the most abundant macroscopic crustacean on sheltered and semiexposed beaches between the Gulf of Maine and Newfoundland (Steele 1976). Its geographical distribution has been described by Croker and Gable (1977). D'Agostino (1980) fed the amphipod *Callinectes laevisculus* (Barnard) to juvenile lobster and obtained wild-type coloration and superior growth rates to those that consumed live *Artemia* and compound diets.

In this trial, growth and mortality rates and coloration of the exoskeleton of juvenile lobsters which consumed live amphipods (*G. oceanicus*) were compared to those that were fed frozen brine shrimp and a compound diet. This compound diet was previously shown to support adequate growth and survival in juvenile lobsters (Gallagher et al. 1979).

### MATERIALS AND METHODS

Amphipods were collected every two weeks from tidal flats at Hancock and Lamoine, Maine. They were most abundant among algal fronds, mussel shells, and under stones. The amphipods were held at a density of 20 to 30/50 cm<sup>3</sup> in perforated plastic freezer containers which contained algal and detrital material within the same system described below for holding the juvenile lobsters.

Postlarval lobsters in stages VI and VIII (obtained from the Department of Fisheries and Oceans, St. Andrews, New Brunswick, Canada) were randomly divided into four groups of 13 to 18 individuals. In Experiment 1, frozen brine shrimp and live amphipods were fed to lobsters maintained individually in perforated freezer containers (7.5 × 7.5 × 10 cm). In Experiment 2, live amphipods and the compound diet were fed to lobsters maintained in similar freezer containers which were divided diagonally to increase holding capacity. The trials began in late July and ran for 96 and 117 days, respectively. Lobsters were fed compound and *Artemia* diets *ad libitum* and excess food was removed daily. Live or injured male and nonovigerous female brine shrimp between 7 and 16 mm were fed as required, one per lobster. Lobsters were starved for seven days prior to the trial.

The lobsters were maintained at Tidal Falls Lobster Pound, Hancock, ME, in ambient seawater temperatures of 8 to 13°C. Mortality and individual weights were recorded at intervals during the trial.

Frozen *Artemia* and their approximate analysis were supplied by San Francisco Bay Brand® (Metaframe Corporation). The percent composition of the compound diet is shown in Table 1 as described by Bayer et al. (1980). The approximate analysis was calculated from literature values (Jurgens 1974). Amphipods were collected on 28 January 1978 for a routine approximate analysis in the laboratory.

TABLE 1.

Percentage composition of the compound diet (Bayer et al. 1980) compared with live amphipods as a diet for juvenile lobsters in Experiment 2.

Ingredient	Percent
Fish meal	30
Yeast	10
Alfalfa	10
Flour	47
Kelp meal	3

At the termination of the trial the lobsters were immediately frozen at  $-15^{\circ}\text{C}$  before six lobsters from each diet were randomly selected. These lobsters were evaluated for exoskeletal pigmentation and coloration by a panel of 11 people. On a scale of 1 to 5, 5 was given to the lobsters with the darkest, red-brown pigmentation.

The "Student's" *t*-test was used to analyze data for significant differences in mean weight gains and mortality for each group, and a comparison of regression lines (Zar 1974) was used to test for significance between growth rates. Significant differences in mean scores for the evaluation of exoskeletal pigmentation were analyzed using Duncan's multiple-range test (Little and Hills 1977).

## RESULTS

In Experiment 1, there were no significant differences ( $P > 0.05$ ) in the mean weight gains or growth rates for lobsters that were fed live amphipods and frozen brine shrimp. Survival was 100% for both groups (Table 2). In Experiment 2, for lobsters that were fed live amphipods or the compound diet, growth rates and mean weight gains were not significantly different ( $P > 0.05$ ), but mortality

was significantly higher ( $P > 0.01$ ) for lobsters that consumed the compound diet (Table 3). All mortalities occurred in the fifth week of the trial. Because death did not occur during the molting phase, mortalities probably were not related to molt-death syndrome. The general trends were for faster growth rates and greater mean weights in lobsters that consumed amphipods in both experiments and, in Experiment 2, for faster growth rates in lobsters maintained in divided containers.

The mean score for the evaluation of exoskeletal color and pigmentation are shown in Table 4. The three diets had a significant effect on the coloration and pigmentation of the lobsters. Those which consumed amphipods had a deep red-brown color and a mean score of 4.51 out of a possible 5. In contrast, those that consumed the compound diet lacked pigment and were grey in color (mean score = 1.55). Those that were fed *Artemia* were intermediate in color (mean score = 2.64). They did not have the deepness of color found in those lobsters that consumed amphipods.

The relative values of live amphipods, frozen brine shrimp, and compound diet as food are compared in Table 5. The compound diet provided a higher concentration of nutrients, particularly protein fiber and carbohydrate. It was impossible to predict the daily intake of each diet because preferences for the diets were unknown, and while uneaten segments of amphipod were rarely seen in the container, significant amounts of uneaten compound diet and brine shrimp were removed daily. On a dry-matter basis, the amphipods and brine shrimp were richer in protein (44.4 and 50.2%, respectively) than the compound diet (34.4%), but lower in carbohydrate (16 and 17.2%, respectively) than the compound diet (42.0%). On a dry-weight basis, amphipods contain 15% lipid; that is considerably richer in fat than the 5.1 and 2.4% in the compound diet and brine shrimp, respectively.

TABLE 2.

Percent survival, growth rates, and mean weight gains for juvenile lobster fed live amphipods and frozen brine shrimp for 96 days.

Diet	Mean Weight (g)			Growth Rate g/10 days	N	% Survival
	Initial	Final	Gain			
Amphipods	$0.290 \pm 0.08$	$0.501 \pm 0.12$	0.211	0.0220	15	100
<i>Artemia</i>	$0.316 \pm 0.12$	$0.485 \pm 0.11$	0.169	0.0176	13	100

TABLE 3.

Percent survival, growth rate, and mean weight gain for juvenile lobster fed live amphipods and compound diet for 117 days.

Diet	Mean Weight (g)			Growth Rate g/10 days	N	% Survival
	Initial	Final	Gain			
Amphipods	$0.275 \pm 0.09$	$0.624 \pm 0.28$	0.349	0.0298	16	100*
Compound	$0.241 \pm 0.07$	$0.493 \pm 0.10$	0.252	0.0215	18	66†

\*† Data indicate a significant difference at  $P < 0.01$ , Duncan's multiple range test.

TABLE 4.

Evaluation for exoskeletal coloration and pigmentation of lobsters fed three different diets.

Diet	Mean Score*	Standard Deviation	Individuals on Panel	Lobsters on each Diet
Amphipod	4.51 <sup>1</sup>	2.12	11	6
Artemia	2.64 <sup>2</sup>	1.62	11	6
Compound	1.55 <sup>3</sup>	1.24	11	6

\*On a scale of 1 to 5, a maximum of 5 was scored for the deepest red-brown pigmentation.

<sup>1,2,3</sup>Data indicate a significant difference at  $P < 0.01$ , Duncan's multiple range test.

The smaller juvenile lobsters were observed capturing and consuming amphipods of almost comparable size. Lobsters would stalk the amphipods as soon as they were placed in the container, but it would take from 1 to 24 hours before they captured their prey. Injured and less active amphipods were captured more readily, but lobsters were uninterested in feeding on immobile or dead amphipods.

#### DISCUSSION

The slow growth rates were probably a consequence of the low ambient seawater temperatures during the trial period, because growth rates of 0.019 g/day for juvenile lobsters that consumed frozen brine shrimp have been reported at 20 to 22°C (Capuzzo 1980). Slow growth rates, together with the large standard deviation in individual weights among each group of lobsters, could account for the lack of significant differences in mean weight gains and growth rates for lobsters that were fed different diets. It is unlikely, however, that any of the diets were quantitatively deficient in either protein, fats, or carbohydrate.

Shleser and Gallagher (1974) found that *Artemia salina* supplied all of the nutrients required for the general health and growth of juvenile lobsters; however, Castell and Budson (1974) obtained the best growth with diets containing more than 60% protein. The latter workers found that the protein-energy ratio was more important than absolute amounts and suggested an optimal ratio of 0.07. The comparable ratios for the compound diet, amphipods, and *Artemia* were 0.08, 0.11, and 0.17, respectively. The nutritional analysis of amphipods collected on 28 January 1980

occurred at a time of low nutrient availability. The nutritive value of amphipods probably varies seasonally as there is a reciprocal variation in protein and lipid levels among many crustaceans throughout the year (related to both maturity of the individual and food abundance). Variations in lipid levels are more dramatic in some marine zooplankton with almost 50% reductions in lipid levels in winter when food is scarce (Raymont et al. 1971, Percy 1979). A lower protein-energy ratio, with a value closer to the optimal protein-energy ratio (0.07), could be expected, therefore, during the period of the trial from late July to September when food for amphipods was abundant.

Growth rate and survival are probably closely related to a correct balance of essential nutrients and deficiencies in the diet. The success of the amphipod diet may be related to its live state, thereby providing a source of unaltered nutrients. The high temperatures used in processing compound feeds and the packaging and freezing of brine shrimp may cause the deterioration of nutrients. Further losses result from leaching of water-soluble nutrients, particularly vitamins, once the diet is immersed. These effects may have led to deficiencies that accounted for mortalities in those lobsters that were fed the compound diet.

A live, algal-detrital feeder such as *G. oceanicus* has additional dietary advantages. Particulate organic matter and fouling organisms that normally accumulate in holding containers and may cause a bacterial buildup and impede water flow are consumed by live food organisms. The amphipod exoskeleton may also form an important source of calcium and phosphorus for freshly molted lobsters. The dietary requirements for these minerals increases following molting (Leavitt 1977).

The predominant invertebrate carotene found in amphipods is astaxanthin and its oxidized product astacene (Sorenson 1936, Goodwin 1960). More recently,  $\beta$ -carotene, canthaxanthin, lutein, and a number of  $\gamma$ -carotene and  $\gamma$ -carotene derivatives have been identified (Czeczuga 1970, Czeczuga and Skalski 1973). The main plant pigment,  $\beta$ -carotene, and other plant xanthophylls are available to the lobster as undigested plant material in the amphipod gut. The presence of the astaxanthin-protein complex gives the characteristic coloration to the lobster exoskeleton. Although the lobster may absorb astaxanthin and other carotenoids through the gut and utilize them directly,

TABLE 5.

Relative analysis of diets as fed in Experiments 1 and 2 to compare their effects on growth and survival rates and the exoskeletal coloration in juvenile lobsters.

Diet	Composition of Wet Weight (%)						
	Moisture	Crude Protein	Lipid	Ash	Fiber	Carbohydrate	Protein/Kcal/g
Compound <sup>1</sup>	0.3	33.40 (34.4) <sup>4</sup>	4.90 ( 5.1)	8.12	0.60	40.80 (42.0)	0.082
Artemia <sup>2</sup>	90.0	5.02 (50.2)	0.24 ( 2.4)	2.92	0.29	1.72 (17.2)	0.172
Amphipods <sup>3</sup>	77.5	9.98 (44.4)	3.36 (15.0)	5.31	1.55	3.85 (16.0)	0.112

<sup>1</sup>Bayer et al. 1980 (calculated)

<sup>2</sup>San Francisco Bay Brand, Newark, CA

<sup>3</sup>*Gammarus oceanicus*

<sup>4</sup>Dry matter basis

Katayama et al. (1971) demonstrated that a number of crustaceans, including the spiny lobster *Panulirus japonicus* Siebold, have the ability to metabolize astaxanthin from  $\beta$ -carotene via a pathway that includes isocryptoxanthin, echinenone, canthaxanthin, and 3-hydroxycanthaxanthin, respectively.

Reports on the predominant carotenes in *Artemia salina* are conflicting. Krinsky (1965) and Hata and Hata (1969) found that canthaxanthin was most important while Davies (1970) found that  $\beta$ -carotene was dominant. None of these workers found any astaxanthin; however, Gilchrist and Green (1960) found that astaxanthin was the dominant carotene and suggested that the presence of carotenoids is related to the food eaten. Carotenes and xanthophylls are very susceptible to breakdown when exposed to light, oxygen, and high temperatures. Peterson et al. (1966) detected a 19% decrease in the levels of xanthophylls in crayfish extract when frozen in the dark for four weeks. Pigment decomposition and perhaps low levels of astaxanthin in the *Artemia* diet may account for the lower coloration score of lobsters fed either brine shrimp or compound diets.

Amphipods may be an abundant, untapped source of food for a variety of commercially important aquatic animals in addition to juvenile lobsters. They show remarkable geographical diversity. Species are found in freshwater lakes and rivers, estuaries and salt marshes, and throughout the world oceans from the Arctic to the Antarctic.

The selected amphipod species should, therefore, survive and reproduce under the same environmental conditions as the farmed organism and/or be harvested locally. In many Canadian lakes, for example, the amphipod *Gammarus lacustris* (Sars) is the most important food organism (>95%)

for the rainbow trout *Salmo gairdneri* (Richardson), and it is responsible for the pink color in the flesh of the trout. Those lakes are potential sites for the commercial farming of rainbow trout (Johnson et al. 1971).

Techniques have been developed for mass culture of amphipods in the Caspian Sea (Zubchenko 1975). In Maine streams amphipods have been caught in elver nets in quantities of 25 kg per hour (R. F. Alvarez, Lamoine, ME; pers. comm.). Reproductive populations can be maintained under laboratory conditions (Nilsson 1974, Steele 1976, Parsons and Bawden 1979) and could be grown within the same system as the farmed species if algal and detrital material were provided. Cultured amphipods could then improve the coloration of the farmed species.

Shrimp meal is often incorporated into the diets of intensively reared trout to increase the pink coloration of the flesh and to improve the taste and odor of trout (Saito and Regier 1971, Steel 1971). In Japan, shrimp meal enhanced acceptance when fed to the freshwater prawn *Macrobrachium rosenbergii* (de Mar) and improved the color of cooked meat. Amphipods that were fed as a live supplement or incorporated in the compound diet may serve as an alternative source of carotenoids and have similar effects on the appearance, taste, and odor of intensively cultured organisms.

#### ACKNOWLEDGMENTS

The authors thank Herb and Pat Hodgkins for the use of their facilities at Tidal Falls Lobster Pound and Bill Barter for help in caring for the lobsters. This work was funded in part by NOAA Sea Grant No. NA81AA-D-00035 and the Maine Agricultural Experiment Station.

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# JOURNAL OF SHELLFISH RESEARCH

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December 1982

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**COVER PHOTOGRAPH:** A large Atlantic surf clam, *Spisula solidissima* (Dillwyn) (Bivalvia; Mactridae), from the eastern coast of the United States. This clam species supports a large commercial fishery along the coast from New Jersey to Virginia. The maximum recorded shell length for the species is 22.6 cm. [Photograph provided by John W. Ropes, Northeast Fisheries Center, National Marine Fisheries Service, Woods Hole Laboratory, Woods Hole, Massachusetts.]









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